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ERRATA AND AUTHORS' EMENDATIONS

Page 9, line 23, "spikelet" should read "group."
Page 16, line 36, "*Triticum*" should read "*Agropyron*."
Page 68, after line 22 insert "picture."
Page 84, last line, omit "Not seen."
Page 124, line 23, omit "15."
Page 140, legend of Plate 15, omit "Late rosette stage of Chinese colza seedling."
Pages 166, 168, 170, 175, 178, 180, and 181, Tables II, IV, VI, IX, XI, XIII, and XV, after column heading "ash" insert "expressed as percentages of dry matter."
Pages 183 and 184, Tables XVI, XVII, and XVIII, column 2, omit "per cent."
Page 193, line 16, "epidemic" should read "endemic."
Page 200, line 23, "0.005" should read "0.0005."
Page 236, footnote to Table XII, after "42 days" insert "and 60 days."
Page 246, line 23, after "apical portion" insert "of claval region."
Page 413, Table I, column 10, footnote reference "e" should read "f."
Page 414, Table I, column 6, footnote reference "o" should be transposed to column 7.
Page 422, citation 4, omit "In press" and insert "no. 9, p. 235–243."
Page 452, Table II, line 1, footnote reference "a" should be inserted before all entries.
Page 479, line 32, "organism" should read "organisms."
Page 481, "26°" should read "25°."
Page 491, "Table XVII" should read "Table XVIII."
Page 508, Table I, column 3, lines 13 to 22, ".283" should read ".028."
Page 607, line 13, "molas" should read "molar."
Page 614, Plate 72, figure E, and Plate 74, figure E, "ae" should read "al."
Page 810, line 25, "Kostelezkya" should read "Kosteletzkya."
Page 815, line 32, "divini" should read "diveni."
Page 816, line 18, "hessitans" should read "haesitans."
Page 822, line 38, "Kosteleyzkya" should read "Kosteletzkya."
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FUSARIUM-BLIGHT (SCAB) OF WHEAT AND OTHER CEREALS[1]

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INTRODUCTION

The cereal crops—wheat, spelt, rye, and oats—and also some grasses are subject to attack by a number of fungi belonging to the genus Fusarium, of which the most common and most important is known, in its ascigerous form, as *Gibberella saubinetii* (Mont.) Sacc. The organism attacks each of the hosts named above in at least two different ways, producing two distinct pathological conditions. The first condition results from an attack on the root systems and the bases of the young and later of the grown plants, occasionally causing partial or entire wilting. The second condition results from an attack upon some of the parts above ground. This may be a rotting of the nodes, found on rye, wheat, and barley, or blighting of the heads of wheat, spelt, rye, barley, and, less commonly, of oats and certain grasses. In all cases the various attacks on the same host are independent of each other. A wheat plant may be attacked underground or on the head only or on both the roots and the head, and in some cases even on some of the nodes; but in all cases these infections are quite independent.

Up to the present time little attention has been given to these two forms of attack by Fusarium, and they have commonly been considered two different diseases caused by one or more unknown species of Fusarium.

However, the results of the work reported here prove conclusively that these two conditions are only two different phases of the same problem. This is in accord with views previously held by Selby and Manns (*11*),[2] Schaffnit (*8*), and Naumov (*5*).

This report, which is of a preliminary nature, deals primarily with the headblighting of wheat, spelt, rye, barley, and oats, as caused by *Gibberella saubinetii*, comparatively little attention being given in this paper to the rootrot caused by this organism. Nothing will be said

[1] In cooperation with the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture.

[2] Reference is made by number (italic) to "Literature cited," p. 31-32.

here concerning other species of Fusarium connected with both phases of this problem or of their possible relation to the similar diseases of corn. While *G. saubinetii* is unquestionably the cause of headblighting of the cereal crops under most conditions and throughout the greater part of this country, it is equally true that under certain conditions and in some parts of the country other species of Fusarium are also responsible for the headblighting of cereal crops. The following organisms besides *G. saubinetii* have been isolated from blighted wheat, rye, oats, and barley heads or plants: *Fusarium avenaceum* (Fr.) Sacc., *F. herbarum* (Corda) Fries, *F. culmorum* (W. G. Smith) Sacc., *F. culmorum* (W. G. Smith) Sacc. var. *leteius* Sherb., *F. arcuosporum* Sherb., *F. scirpi* Lamb et Fautr., *F. solani* (Mart. pr. p.) Ap. et Wr., *F. arthrosporioides* Sherb., and *F. redolens* Wr. These species, while very seldom responsible for the headblighting of cereals, are not so unimportant in the rootrot problem of these crops. Indeed, some of them (*F. herbarum*, *F. avenaceum*, *F. culmorum*, and *F. culmorum* var. *leteius*) have, in my observations, proved to be as important as *G. saubinetii* in causing rootrot of the cereal crops.

There is extensive literature on this subject which can not be reviewed in this brief paper. Only a few of the more important citations are given.

THE DISEASE

COMMON NAME

In this country the headblighting of the cereal crops is generally known under the faulty name of "wheatscab." It is not a wheat disease alone, because it also occurs on spelt, rye, barley, oats, and certain grasses. And it is not "scab" because it causes no scabbing of the heads or of any part of the various hosts but rather blighting of the heads. The infected heads are perfectly normal and remain so except that they are blighted, take on the color of bleached straw, and later may be overgrown with the mycelium of the pathogen. Since the name "wheatscab" is faulty in a number of respects, the name "Fusarium-blight" is used in this paper.

GEOGRAPHIC DISTRIBUTION

The Fusarium-blight of cereals is more or less common throughout the central and eastern cereal-growing sections of the United States. It has been reported by the Plant Disease Survey for 1917, 1918, and 1919 from the following States: Maine, New Hampshire, Vermont, Massachusetts, Connecticut, New York, Pennsylvania, New Jersey, Delaware, Maryland, West Virginia, Virginia, North Carolina, South Carolina, Georgia, Alabama, Tennessee, Kentucky, Ohio, Indiana, Michigan, Illinois, Wisconsin, Minnesota, Iowa, Missouri, Oklahoma, North Da-

kota, South Dakota, Montana, and Oregon. It was looked for but was not found in the following States: Washington, California, Wyoming, Texas, Arkansas, Kansas, Louisiana, Mississippi, and Rhode Island. It has been reported from various parts of Canada.

In Europe the disease has been found in England, France, Italy, Germany, Austria, Holland, Denmark, Norway, Sweden, and Russia. In Russia the disease is common throughout the wheat- and rye-growing sections. In Asia it is very common in the Usurian provinces on the Siberian Pacific coast. It has also been reported from Australia.

ECONOMIC IMPORTANCE

The Fusarium-blight of the cereal crops injures the plants in several ways and is generally considered an important disease of these crops. It lowers germination of the seed and causes dying off or weakening of the young seedlings. Later it causes dying and wilting of fully grown plants, and finally it blights the heads, wholly or in part, thus preventing them from filling. The severity of the headblighting varies from a fraction of 1 per cent to 100 per cent, and the loss due to decrease in yield in individual fields and localities may vary from 0 to over 50 per cent.

The data concerning the economic importance of the disease are incomplete and inadequate. For some phases of the disease, and for most of the crops, they are entirely lacking. The meager information at hand on this subject is found in the Plant Disease Bulletin issued by the United States Department of Agriculture.[1] This covers only the losses caused by blighting of the heads of wheat and is given for only a few of those States where the disease is known to be present and common in one form or another. No information is available concerning the losses due to decrease in germination and the killing of seedlings and grown plants.

The total loss due to the blighting of the wheat heads by *Gibberella saubinetii* and various other species of Fusarium for the States reporting amounted to 10,620,000 bushels in 1917, according to the Plant Disease Survey. The States reporting highest losses were Ohio with 3,577,000 bushels, Indiana with 2,513,000 bushels, and Illinois with 2,288,000 bushels.

If the estimate of the Plant Disease Survey approximates the actual loss due to the blighting of the wheat heads in the States reporting, then the total annual loss for the United States is probably close to 20,000,000 bushels.

No definite information is available concerning the importance of the disease in Europe, especially in Russia, where it is known to be one of the most important and destructive of the cereal diseases.

[1] U. S. DEPARTMENT OF AGRICULTURE. BUREAU OF PLANT INDUSTRY. PLANT DISEASE SURVEY. PLANT DISEASE BULLETIN, SUPPLEMENT 8, p. 21-27. May 1, 1920.

DESCRIPTION

In spite of the extensive literature on this subject, there is no detailed description of any of the various phases of the disease. In some discussions of the disease no symptoms are given; in others there is a brief description of only the last stages of infection, or rather, of the final results of infection. Because of this situation it seems necessary to describe the disease in detail, giving special attention to some symptoms which previously have been overlooked.

BLIGHTED SEED

Wheat kernels obtained from heads blighted or partly blighted by *Gibberella saubinetii* show marked evidence of the effect of the Fusarium attack and can be easily distinguished in a sample of grain, even when only a very small percentage of such kernels are present. Wheat seed from blighted heads exhibits one of three more or less distinct and definite pathological symptoms, depending upon the time of head infection.

(1) Kernels from heads infected early in their development, possibly during or shortly after the blossoming period, are small in size, being sometimes hardly two-thirds as long as the normal. They are pale greenish gray in color, badly shrunken, not firm, and very light in weight. As a rule, such kernels are never able to germinate. They may be heavily infected or even covered with the mycelium of the fungus if they developed near the point of infection, or they may be perfectly free from any fungus mycelium, if they have developed far above the point of infection where the food supply was cut off.

(2) Kernels from heads infected two or three weeks after the blossoming period may attain nearly a normal size, but they usually have a slightly shrunken appearance. They are grayish white or cream-white in color, soft and starchy in texture, and much lighter in weight than the normal kernel. In this case, also, they may be infested and even covered with mycelium, which is especially evident in the groove, or they may be entirely free from mycelium, depending on their position in the head with relation to the point of infection. The percentage of germination of kernels in this class is very low.

(3) The third class of kernels consists of those which have been infected shortly before or just after the head is ripe. Such kernels differ very slightly from the normal, except that they are partly discolored, pinkish spots being not uncommon on them. While it is true that *Gibberella saubinetii* is the most common cause of pinkish red coloring on kernels in all three of these classes, it must be remembered that other fungi, Macrosporium and Alternaria for instance, may in some cases cause this coloring of grain. Kernels of this last class usually germinate normally, but before the young plant has reached the surface of the soil, or before it attains any considerable size, it not uncommonly wilts and

dies as a result of infection from the kernel. In many cases, however, the seedling survives the attack and reaches full development.

Kernels of rye from blighted heads show symptoms similar to those described for wheat. The kernels which are directly attacked by the fungus in blighted barley heads become dirty brown in color and are lighter in weight than the normal kernels if the infection takes place at an early stage in development. Often barley kernels are found with salmon-colored spots on which there are masses of conidia of Fusarium. Oat kernels show much the same symptoms as barley, except that they remain lighter in color. In all these cereals, symptoms similar to those caused by Fusarium-blight may be caused by other agencies, such as the exposure of the grain to rain.

SEEDLING-BLIGHT

Seedlings from seed naturally or artificially infected with *Gibberella saubinetii* are subject to attacks by this organism at a very early stage of their development, and the visible symptoms of the infection may become evident at the time of the germination of the seed or only a few days later. The first symptoms appear on the young coleorhiza and coleoptile and consist of the browning and rotting of these parts. The coleorhiza and coleoptile, which always die shortly after the formation of the permanent roots and the appearance of the first foliage leaf, seem to offer a good medium for the establishment of the various species of Fusarium, which then penetrate into the tissues of the permanent roots and the first foliage leaf, causing the browning and rotting of the invaded portions. If the attack has proceeded successfully, the formation of the two lateral roots, in the case of wheat, is either prevented or these roots are destroyed before attaining any considerable size. The older or basal portions of the roots are sometimes pink in color, but they are usually brown to black. The lower portions of the roots continue normal and healthy until their food supply is cut off by rotting of the upper parts. Often the remnants of the kernel are heavily overgrown with the mycelium of the fungus, and in some cases they attain a dark carmine red color. The leaves above the infected portion, which seldom extends above the ground if the plant is still very small, become yellow and later brown, the discoloration beginning at the tips. If the leaves are over 6 cm. long they usually take on a light-green color and then collapse and wilt very rapidly, showing a blighted effect. In many cases the infection may be restricted to the primary roots, the coleorhiza and coleoptile, and even to the first foliage leaf. In such cases new roots are soon formed, the second and third leaves develop, and the plant may recover almost entirely from the attack, which is still restricted to the parts originally infected. Such plants, if examined three or four weeks later, will show no symptoms of the infection and will usually continue to develop normally.

FOOTROT

Careful examination of the underground portions of winter crops early in spring and of spring crops somewhat later in the season shows partial rotting of the roots, the bases, and, in some cases, the interior of the stems just above the bases. Various fungi may be found associated with this condition on the cereal crops, among which *Gibberella saubinetii* and species of Fusarium are common. No attempt has been made to obtain definite data on the relative frequency of occurrence of different species of Fusarium on root lesions and discolorations. This, of course, would be necessary before their relative importance as organisms inducing rootrot under field conditions can be determined.

The first evidence of the pathological condition of the roots of the cereals, whether the source of infection be the seed or the soil, is the same. The organisms first appear on the remnants of the kernel and follow some of the primary roots, causing rotting and browning as described above. When the crown and the crown roots are formed, the primary stem below the crown roots, now quite darkened and in some cases beginning to die, is invaded by the organism from the remnants of the kernel and the primary roots. Soon it, too, becomes brown and shows evidence of rotting. When the invasion reaches the crown it may stop, or, depending perhaps on the condition of the plant, it may continue, invading the central woody portion of the primary stem above the crown as well as the secondary stems and causing a browning of the woody portions. Rotting and browning of the scale leaves and of the sheath may also occur as a result of the invasion. How much of this rotting and discoloration of the underground portion of the cereal crops due to Fusarium species is parasitic and how much is saprophytic is not known. That some of these organisms are parasites is shown conclusively by the rotting of the roots next to the remnants of the kernel or next to the crown while their lower portions continue to be normal. It is shown also by the browning of the interior of the primary stem at and above the crown. The separation of discolorations and rotting of underground portions due to the parasitic and saprophytic action of the organisms concerned is unusually difficult, as large portions of the original underground parts of the plants eventually die even without any fungus invasion, and the presence of parasitic organisms may have nothing to do with it. Such is the case with the primary roots and the primary stem below the crown, and later with some of the crown roots themselves.

The amount of damage, if any, due to this invasion of the roots and other underground portions is even more difficult to determine. As a rule, the plants so attacked are at first small and stunted, but with the coming of sunny and warmer weather they usually recover and reach normal development, even when very badly injured. With the coming of favorable weather such plants may send out secondary roots or even

aerial roots, a development quite common in oats, and before long the effects of the attack may largely disappear.

ON STEMS OF GROWN PLANTS

Occasionally full-grown plants are killed by *Gibberella saubinetii* or by one of several Fusarium species just before or shortly after the time of blossoming. The fungus attacks the roots and the stem close to the ground, the first node usually being involved in the infected area. The part of the stem in contact with the ground and the roots below are rotted and are commonly pink or yellowish brown in color. This rotting of the base interferes with the water and food supply of the plant, and wilting of the entire plant is the result. Such plants become bent or broken over soon after they wilt and hence are easily recognizable in well-kept fields. When such plants are pulled up they break at the base, the roots always remaining in the soil (Pl. 2, A). It must be remembered, however, that wilting of the whole plant in very much the same way is caused by other fungi as well, for example by *Colletotrichum* sp., although in attacks by this fungus the base of the dead plant is a much darker brown or black in color.

This infection at the base of the plant may be due to any one of several causes. It may be only a continuation of the attack upon the young seedling or it may be the result of a new infection. Either the decline in vigor or unfavorable weather conditions may be responsible for the appearance of the disease at this time.

The succulent embryonic tissue just above the nodes of the various cereals is especially susceptible to attack by *Gibberella saubinetii*. Here the infection is usually restricted to the node or the area immediately next to the node, seldom, if ever, extending more than 2½ cm. in each direction. In such cases the portion above the infected node usually wilts and soon dies. Conidia may be formed under certain conditions on the node itself and on the infected part of the sheath coming out from it. This condition was first observed by McAlpine (*4, p. 305*) in 1896.

BLIGHTING OF HEADS

WHEAT.—The symptoms and effects of headblighting of different varieties of wheat are, in general, the same. The blighted head usually takes on the normal color characteristic of the ripe head of that variety or a slightly lighter color.

Blighting of the wheat heads can be detected with absolute certainty at a very early stage, three to four days after infection has taken place, provided that weather conditions have been so favorable as to enable the parasite to establish itself on the host and to begin its work of destruction.

The symptoms of blight infection as they appear on Marquis or some other of the beardless varieties are as follows: The very first sign of blight

infection is a slightly brown and water-soaked spot, 2 to 3 mm. in length, on the glumes. The veins appear more water-soaked and have a much darker olive-green appearance than the area between them. The points at which the infected glume or glumes are attached to the rachis soon show the water-soaked appearance also. The water-soaked area increases more or less rapidly, depending on weather conditions, until the whole spikelet is covered. It then spreads to the neighboring spikelets.

If the weather is dry the infection may remain restricted to one spikelet. At this time the glumes and the spikelets originally infected gradually begin to lose the water-soaked appearance, dry up, and take on the typical color of the ripe head of the particular variety. This drying up of the infected spikelets follows closely the advancing infection, which usually proceeds downward, as was first observed by Freeman (*2, p. 310*) in 1905. The healthy part of the head above the point of infection usually dries up and dies without passing through the water-soaked stage, because of the cutting off of the water and food supply by the fungus at the point of infection. In some cases, however, one or more vascular bundles of the rachis may remain free from the fungous invasion and continue to supply the uninfected portion of the head with water and food until the head has ripened normally and has formed fairly normal kernels. When infection proceeds down the stem, producing the same symptoms as on the head, it may sometimes reach as far as the upper node. Here, too, the whole or only one side of the stem may become affected, while the other side with one or more vascular bundles still normal may continue to provide moisture and food for the living portion of the head. Usually, however, especially in dry weather, the infection is restricted to the head; and most commonly only a part of the head is destroyed. This may be the upper, middle, or lower part, depending on the kind and point of infection. Infection of the rachis causes blighting or dying of the whole head above the point of infection. In such cases the dead spikelets shrink and become more closely appressed to the rachis, while the uninfected portions of the head continue their normal development to maturity and become robust, with spikelets well filled, thus making the difference between infected and uninfected parts still more striking.

The point of infection, even when the attack is in an advanced stage, can easily be located, especially if the weather has been favorable. It is usually covered at first with a short, cottony, slightly pinkish fungous growth, while the rest of the infected area remains free from such a growth. Later, if the weather is favorable, this growth extends farther over the infected area and becomes the substratum on which a layer of conidia develops. This layer of conidia may be smooth (pionnotes) or more or less granular (sporodochia), depending on the causal organism and the age of the infection. The older it is the smoother it becomes. The conidial masses, which were originally slightly pinkish, now become dark salmon to grenadine in color, depending on the causal

organism. The conidial masses tend to be more dense in the cases of infection by *Fusarium herbarum* and *F. avenaceum* and less so in the case of infection by *Gibberella saubinetii* and other Fusarium species. Because of the fact that at the bases of the spikelets moisture from rain or dew is held for a considerable length of time, the conidia are usually formed here, extending along the furrow formed at the line where the inner and outer glumes meet. In cases where the infection extends down to the upper node, conidia may be produced on the node also. They never form pionnotes but usually produce small sporodochia, which are generally abnormal in size and shape.

RYE.—The symptoms of headblighting of rye are very much like those of wheat, except that the water-soaked appearance is not so prominent. The infection seldom extends as far down as the second node before the plant naturally matures. Conidia are usually formed only at the bases of the spikelets and in the furrow formed where the inner and outer glumes meet and, to some extent, under the outer glumes. In moist weather, however, conidia may be formed throughout the infected area. Heads infected and killed at an early stage remain straight, while normal heads are slightly bent.

BARLEY.—The symptoms of blight on barley heads are usually different from those on wheat and rye, seldom resembling those on the latter. Usually only one kernel is killed, or occasionally several kernels in one row. In some cases the three kernels forming a spikelet are attacked and later, if conditions are favorable, the rest of the head is blighted. The first sign of infection is a small, water-soaked, somewhat brownish spot appearing at the base or the middle of the glume or on the rachis. The water soaking and browning spread in all directions from the point of infection, soon including the whole glume, the whole spikelet, or several spikelets, but the infection is by no means as uniform as it is in wheat and rye.

OATS.—The symptoms of headblighting of oats resemble those of wheat. Because of the structure of the panicle, however, the infection is usually restricted to one spikelet and is therefore not so conspicuous as it is in wheat or rye.

LIFE HISTORY OF THE CAUSAL ORGANISM IN RELATION TO PATHOGENESIS

The life history of the parasite, so far as it is connected with that of the hosts, has been followed by the writer through the entire year, and is here briefly outlined.

PRODUCTION OF SPORES

CONIDIA

Production of conidia upon the host plant is more or less common in all forms of Fusarium attacks on cereals. In many cases it may be so abundant that it leaves no doubt as to the real source of inoculum for subsequent infection in nature.

ON SEEDLINGS.—When a wilted seedling is pulled out and portions of its partly decayed kernel or of the young stem are examined under the microscope, a great number of normally developed conidia can frequently be seen. In rare cases masses of conidia are also formed on the rotted stem above the ground. The number of conidia so formed will be still greater if any particles of organic matter like straw, old stems, or stubble happen to be near the wilted or heavily infected plant, since the conidia-forming growth will extend over them. This growth soon disappears, however, leaving no evidence of its existence.

ON NODES AND BASES.—Formation of conidia on the infected nodes or bases of mature plants, while common, is never very abundant because of the rapid drying out of these parts.

ON HEADS.—The formation of conidia on the heads of cereal crops, especially of wheat and rye, shortly after infection takes place is common and so abundant as to give them a very distinct pinkish or salmon color. In dry weather the formation of conidia is restricted to the area where the infection originally took place, this being usually the base of the spikelet where the rain drops collect and the moisture is held for a longer time than on any other part of the plant, except possibly in the sheaths. The spore formation under such conditions extends up the several furrows formed by the joining of inner and outer glumes and to some extent even between the glumes. In moist weather the conidia are formed in great abundance over the entire surface of the tissue through which the hyphae of the parasite extend. The latter send out conidiophores through the stomatal openings, forming at first small balls of conidiophores and conidia over each stoma. Soon these balls converge into a uniform layer (pionnotes) of conidia extending over a large portion of the head. The following observation in the field corroborates this fact.

Before June 29, 1918, the weather was dry and there were very few conidia formed on the infected rye heads in the University experimental plots. The last two days of the same month were rainy and comparatively cooler. Following this, conidia were formed in such abundance that all the infected spikelets were practically covered with a layer of conidia which gave them a distinctly pink or salmon color.

Dry, blighted rye, wheat, or barley heads without any conidia also produced conidia in abundance when placed on the ground under a screen and kept moist.

ON DEAD ORGANIC MATTER.—Old straw and pieces of stems and cornstalks in fields where the year before the crop had been heavily infected with the disease were often found to show large pinkish areas bearing numerous conidia, some of which belonged to some of the species of Fusarium which were found parasitizing wheat and corn. This condition was especially common on cornstalks and wheat heads left in the field from the previous year and bearing the perithecia of *Gibberella*

saubinetii, thus confirming results obtained by Hoffer, Johnson, and Atanasoff (*3*) in 1918, when it was demonstrated that the hyphae present in the previously infected heads or cornstalks remain viable till spring, when they form new conidia and thus help the further propagation of the fungus.

ASCOPORES

Whenever the cause of the disease is one of the species having a perfect stage, as is the case with *Gibberella saubinetii*, the perithecia of this fungus are produced in great number on all infected parts, but especially on the pseudo-plectenchymatic structures, on which there has been more or less formation of conidia. Perithecia are formed on seedlings and infected kernels (observed only under greenhouse conditions), on the straw and the heads of the various cereal crops, and on the stalks, sheaths, and ears of corn. The ascospores play an important röle in the life of this organism, since they are likely to resist extreme weather conditions and furnish inoculum for the first infection in the spring.

DISSEMINATION OF SPORES

The experimental work on this subject is limited to a study of the agency of wind, and to some extent of rain, in distribution of conidia. Other factors may also play some röle in the dissemination of conidia and ascospores, but time did not permit a study of other factors.

BY WIND

In a rye field slightly infected with blight, numerous spore traps [1] were placed on stakes in vertical and horizontal positions, some on the ground and some at various heights, ranging from 3 to 8 feet above the ground, and exposed from 12 to 24 hours, then examined under the microscope. The number of *Gibberella saubinetii* conidia caught was very small when compared with the number of spores of other fungi, especially rust spores, that was found on each spore trap. *Gibberella saubinetii* conidia varied in number from none to eight on the traps set closest to the ground and especially on those placed vertically and facing the prevailing wind. Most of the conidia of *Gibberella saubinetii* were caught by the traps set on the ground. The statement that the conidia of species of Fusarium are wind-borne is not new. Saïto (*7*), studying the atmospheric flora of Tokyo, found that Fusarium conidia are carried by the air in small number. The same fact has been reported by a number of other workers.

That the ascospores of *Gibberella saubinetii* are also wind-borne is shown by the following observations in the field. One of the rye fields under observation in 1918, consisting of several acres, was located on

[1] Common microscope slides were covered with a layer of glycerin, or glycerin with some vaseline, and were used as spore traps.

top of a hill. The field, which was only partly in rye, sloped at its west end rather sharply to the south and at the east end sloped gently to the south and east. The north side, the top of the hill, was fairly level and protected by a wind-break of trees. To the east and west also there were trees. The top or level part of the hill was sown with winter rye and the sloping parts with second-year alfalfa in which barley had been the nurse crop the preceding year. On the old barley stems left in the alfalfa field were a considerable number of *G. saubinetii* perithecia with viable spores. The only wind that could reach this field was from the south. The rye field was as uniform as could be expected in all respects except slope. The degree of headblight infection, however, was very different in the different parts of the field, although it was only a small and narrow strip of land. Blight was practically absent in the west part, which was surrounded on the north and west sides by wind-breaks. However, on the southwest edge there was considerable blight infection among the plants that were immediately next to the alfalfa field in which, as stated above, *G. saubinetii* was present and the slope was very steep. The east part of the field, which was protected on the north and east sides by wind-breaks, had, on the other hand, up to 5 per cent of blight, not only among the plants next to the alfalfa field but also throughout its south half, while its north half was free from blight. Knowing of no other factors that could account for this difference, the writer is inclined to think that the following is the possible explanation of the distribution of the disease. The west end of the field bordering on the alfalfa field where the slope was steep was infected only through the area next to this field, because the wind, lifting the spores from the alfalfa field, could not raise them into the upper air currents and so over the hill but deposited them against the slope before they could reach the rye plants on the level ground. Thus, only those rye plants were infected that were next to the alfalfa field. In the east part of the field the situation was different. The slope there was gradual and the spores needed to be lifted only several feet in order to be on a level with the rye field. Thus they could be easily carried to the rye plants even by the slightest air currents; and for this reason, perhaps, the infection in this part of the field was greater, although even here it was restricted to that half of the field which bordered on the alfalfa field. This indicated that the source of infectious material was the alfalfa field and that the infection extended only as far as the topographical conditions permitted the wind to carry the spores.

BY RAIN

The conidia produced at first are usually very loosely attached to the mycelial growth and are easily detached from it by wind, insects, and other agencies, while the conidia formed later and in pionnotes, as is commonly the case, stick together. However, if a drop of water is placed on the pionnotes the spores are set free with great rapidity and

force, as shown by the fact that they are driven around in the drop with considerable velocity. It is rather evident, therefore, that rain assists in the liberation of conidia from the pionnotes, and thus they are carried down to the ground or transmitted from plant to plant as the plants wave in the wind.

Insects, no doubt, may also play some rôle in the dissemination of Fusarium conidia, but time did not permit a study of their importance.

TIME OF NATURAL INFECTION

The first blight infection in nature takes place during the latter part of the blossoming period. It is, however, not the most severe one; the secondary infections following shortly after the first being the ones that are most destructive.

Several wheat, rye, barley, and oat fields, all located within 4 miles of Madison, Wis., were selected for experimental purposes during the spring and summer of 1918 and were examined every other day, beginning about one week before the period of blossoming of rye and two weeks before the blossoming of wheat, barley, and oats.

The following is a typical brief record of the observations on one of the wheat fields:

Station No. 2. Town of Burke, Wis.

Field of Marquis wheat on corn ground. Field in level open country. Soil sandy loam. Stand good.

June 22, 1918. Plants in blossom. No signs of blight infection. Throughout the field there are numerous cornstalks with a great number of *Gibberella saubinetii* perithecia with viable spores.

June 28, 1918. Wheat just passing blossoming stage. No signs of blight infection. Ascospores in masses are oozing from Gibberella perithecia.

July 7, 1918. First indication of blight infection apparent. It consists of a water-soaked spot on single spikelets, usually on single glumes.

July 15, 1918. All suspected first infections have developed into distinct blighting of the heads.

Following the first infection there may be as many successive infections as weather conditions permit.

This observation agrees with the results obtained with artificial inoculations. Inoculation of plants before blossoming and following the dough stage gave negative results. While the organism will attack and penetrate the heads and the kernels in them during the latter part of the dough stage and also after maturity, as demonstrated first by Schaffnit (*8*) and later by Naumov (*5*), if there is abundant moisture and warm weather, this can scarcely be spoken of as infection in the true sense of the word. Wheat plants which were just heading out, others which were just past blossoming, and a third lot which were in the late dough stage were inoculated under exactly the same conditions, on the same day, and with the same spore suspension. They gave the following results: The first and third lots remained healthy during the first

week, while the second lot showed 100 per cent severe infection and the third lot remained free from the disease until full maturity. Some of the plants in the first lot showed slight infection seven days from the time of inoculation, during the time when they were in blossom. These results show that the spores remain on the infected heads until the heads reach a susceptible stage before infection takes place.

SOURCE OF NATURAL INFECTION

An important source of infection is the seed used for sowing. Cereal seeds carry, externally, viable conidia of *Gibberella saubinetii*, as well as of *Fusarium* spp., and many of the kernels are internally infected with these fungi, as has been shown by Selby (*9*), Selby and Manns (*11*), Schaffnit (*8*), Bolley (*1*), Wollenweber (*12*), Naumov (*5*), and many others. Many times the writer isolated *G. saubinetii* and several Fusarium species from what seemed fairly normal wheat, barley, rye, and oat kernels, as well as from kernels from blighted heads of the same crops. In all cases *G. saubinetii* was the organism most commonly isolated. Seed so infected carries the organism to the soil, where it attacks the young seedlings if conditions are favorable. It passes the winter in the soil, preferably on the killed seedlings or other organic matter. In the spring it resumes its growth, producing new conidia which when carried to other parts of the plant cause head or node infection.

The perfect stage of this organism, which is formed in abundance on infected heads, straw, or cornstalks, is an important source of natural infection. The conidia of this organism, which are always produced in abundance on the infected heads and stems, are the chief, if not the only, source of secondary infection.

Whether *Gibberella saubinetii*, as well as the other Fusarium species attacking the cereal crops, is present in the soil at all times and for long periods of time, always ready to attack the susceptible hosts sown on such soils, is an important phase of this problem to which the writer has given no attention.

OVERWINTERING OF THE FUNGUS

The organism, because of its comparative resistance to cold and drying, overwinters in various ways. When introduced into the soil with the winter crops, it overwinters in the form of mycelium and conidia where these are formed on the killed seedlings and on other organic substances. It also overwinters in the form of mycelium in and on the seed, straw, heads, and cornstalks that have been infected with the fungus the summer before. The organism has been isolated from such plant parts kept out of doors throughout the winter and spring. During the winter

of 1918 it was frequently isolated from cornstalks fed to the cattle on the University farm and from cornstalks that had been taken out into the fields with the manure or for cattle feeding.

The mycelium of the organism present in infected straw and heads of wheat, rye, and barley when stored in the laboratory at room temperature and moisture was found viable after 12 months. In the infected seed it remains viable even after the second year.

The undeveloped perithecia of the organism, which are often found in the fall on the straw and heads of the cereal crops, on cornstalks and sheaths, and on many grasses, are another form in which this organism overwinters. In the spring these perithecia mature and form numerous ascospores, which are later liberated from the perithecia and carried to the various susceptible hosts. Mature ascospores in perithecia on wheat heads and cornstalks preserve their viability for over 8 months when kept in the laboratory at room temperature and moisture.

DESCRIPTION OF CAUSAL ORGANISM

TAXONOMY

The chief cause of headblight and one of the chief causes of rootrot of the cereal crops in the United States is *Gibberella saubinetii* (Mont.) Sacc. The following is a list of synonyms:

Gibberella saubinetii (D. and M.) S., 1879, *in* Michelia, v. 1, p. 513.

Gibbera saubinetii Mont., 1856, Syll. Gen. Spec. Crypt., p. 252.

Botryosphaeria saubinetii (Mont.) Niessl, 1872, *in* Verhandl. Naturf. Ver. Brünn, Bd. 10, p. 195, pl. 4, fig. 29.

Fusarium graminearum Schwabe, 1839, Fl. anhalt, v. 2, p. 285, pl. 6, fig. 7; Sacc. Syll. v. 22, p. 1483–1484, 1913.

Gibbera pulicaris (Fr.) f. *zeae maydis*, Rehm: Ascomyceten 381. From New Jersey, 8, 1875, J. B. Ellis.

Fusarium roseum Autorum.

Fusarium tropicalis Rehm, 1898, *in* Hedwigia, Bd. 37, p. 194. Is probably a synonym of *Gibberella saubinetii* according to Wollenweber (*12*).

Gibberella tritici P. Henn., 1902, *in* Hedwigia, Bd. 41, p. 301.

Fusarium rostratum App. and Wollenw., 1910, *in* Arb. K. Biol. Anst. Land u. Forstw., Bd. 8, p. 30.

MORPHOLOGY

PERITHECIAL STAGE.—The following description of the perfect stage of this organism, given by Wollenweber (*12*), is adequate:

Diagnosis.—Perithecial stage: Perithecia scattered or gregarious, ovoid to subconical, free on the surface of the host as well as embedded in mycelium, or on a tubercular plectenchymatic stroma, which may either push in sphaerostilbe-like bodies through

the surface of the host or remain endophytic, 150 to 250 by 100 to 250 μ. Peridium smooth and small-celled at the basal part, but large-celled, verrucose occasionally, with protuberance-like projections of cell groups near the apical end, black to the unaided eye (turning red-brown with acid reaction), dark blue with transmitted light except the almost colorless often rather prominent beak; asci up to over a hundred in each perithecium, intermixed with a few celled paraphyses; ascospores, 8 in one row or irregularly in two rows, subdorsiventral, fusiform, slightly curved, tapering at the ends, ochreous in masses; largely 3-septate, 20 to 30 by 3.75 to 4.25 μ (up to 5 μ in diameter in germination, indicated by constrictions at the septa).

CONIDIAL STAGE.—In shape the conidia (fig. 1) strongly resemble the conidia of *Fusarium culmorum*, but they lack the constriction toward the base so prominent in *F. culmorum*. They differ also in being longer and more slender and in having thinner walls and less prominent septa; conidia typically, sometimes up to 100 per cent, 5-septate, 45 to 65 μ by 4.2 to 5.5 μ; 3-septate, 35 to 45 μ by 5 to 5.5 μ; seldom 4-septate; rarely 6-, 7-, or more septate, 60 to 75 μ by 4 to 5 μ; ochreous in mass. Chlamy dospores absent. Carmine red pigment on starchy, neutral media.

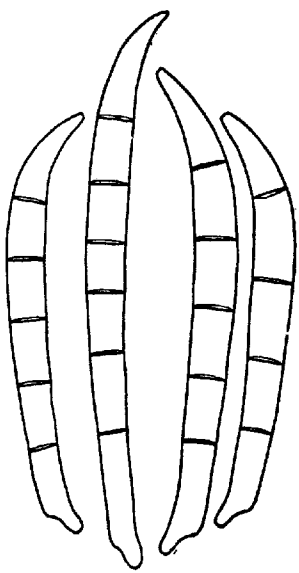

FIG. 1.—Conidia of *Gibberella saubinetii*.

HABITAT.—This species is one of the most widely distributed species of Fusarium within the temperate zone, causing headblight and rootrot of wheat, emmer, rye, oats, spelt, and corn in the United States, Germany, Russia, Italy, Denmark, Sweden, and probably elsewhere. Wollenweber isolated it from berries of *Solanum tuberosum* near Berlin, Germany. C. A. Ludwig isolated the same from *Ipomoea batatas* in storage at La Fayette, Ind. The writer found the perithecia of the fungus on Bromus, timothy stems, clover, and alfalfa, and also on *Triticum repens* which had been plowed under. The fungus was also isolated from asparagus stems collected at Baraboo, Wis., by Mr. E. H. Toole. According to Saccardo (*6, p. 513*), the fungus occurs on dead stems of Angelica, Asparagus, Beta, Clematis, Conium, Cannabis, Convolvulus, Cucurbita, Gyneria, Phytolacca, Scirpus, and Stipa, and on branches of Buxus, Coronilla, Fraxinus, Gleditschia, Juglans, Robinia, Rubus, Rosa, and Ulmus in Europe, Algeria, North America, and Australia. A. D. Selby (*10*) adds Emmer, Trifolium, and Medicago as new hosts. It has been found also on *Glyceria aquatica* in Germany, on rice in Japan and Italy, and on *Triticum spelta* in S. Paulo, Brazil.

METHOD OF PERFECT STAGE DEVELOPMENT

IN NATURE.—A limited study of the field conditions under which the perfect stages of some Fusarium species which parasitize the cereal crops and numerous grasses are formed showed that those conditions are as follows:

(1) Successful parasitism of the fungus on some host. The perithecia are formed usually and preferably on those dead parts of the host which have been parasitized.

(2) Successful conidia production. Conidia production on the infected substratum, root, stems, or heads always precedes the formation of perithecia, since the latter are formed more readily on the crust or plectenchymatic layer formed by the conidia-bearing hyphae and the germinated masses of conidia themselves.

(3) Presence of moisture. No perithecia will ever be formed in the absence of sufficient moisture, and their formation will be delayed until moisture is sufficient.

(4) Suitable temperature also must play some rôle in the formation of the perithecia. Formation of perithecia took place only during the summer when the temperature was highest. Efforts to develop the perithecia from infected material during October and November gave negative results.

When the foregoing conditions were established as factors in the formation of perithecia, the following method of producing them was worked out and has yielded good results. The infected parts of the various cereals, including corn, such as stems, nodes, sheaths, heads, and ears, were gathered from the field and laid on the ground during July and August, 1918, then covered with a wire screen, moistened thoroughly, and covered with some dry grass and leaves to protect them from drying out. During the first and second weeks, masses of conidia were formed over the entire infected area of the various parts. Soon this extended even over the uninfected area. Before long all conidia germinated and no others were formed. During the third week the perithecia began to be formed. In three or four more weeks numerous perithecia were formed, most of them with matured ascospores.

The following is a record of one of the experiments for perfect-stage development:

June 28, 1918. Rye heads infected with *Gibberella saubinetii* were placed under screens so as to be exposed to the action of the weather. They were sprayed thoroughly with water and covered with dry grass to protect them from drying out.

July 16, 1918. First perithecia beginning to appear.

August 2, 1918. Numerous perithecia formed, but asci not yet fully developed.

August 21, 1918. All perithecia have ripe ascospores. Heads taken to the laboratory for study.

IN LABORATORY.—Infected wheat kernels, when placed in a pot filled with fine sand and only slightly covered with sand and kept moist at

room temperature, produced numerous perithecia on their exposed surfaces. These matured before the end of the fourth week from the time of sowing. As soon as the ascospores in the perithecia were found to be mature, the kernels were sifted from the sand and preserved in dry condition until needed for study or inoculation.

The development, in the laboratory, of perfect stages of those species of Fusarium which have a perfect stage was secured in the way originally described by Appel and Wollenweber and later extended by Wollenweber. It need only be emphasized that the perithecia of these fungi will rarely be formed until the transfers and cultural work are begun from what these authors call "normal" culture. Failure is bound to occur 95 times out of 100 before the culture which is to be used for development of the perfect stage is brought to this condition.

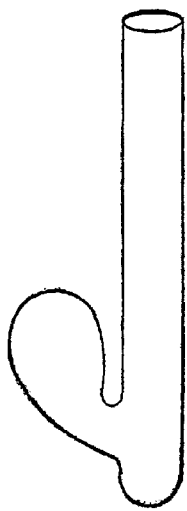

FIG. 2.—Special culture tube for maintaining moisture in culture. × ½.

Once the culture is in the proper condition, the next step consists in transferring it to media that are known to favor the development of the perithecia, such as stems of any kind, but especially those of *Melilotus alba*, bean pods, etc.

Care must be taken that the cultures on *Melilotus alba* stems or other media are kept uniformly moist until the perithecia are formed and the ascospores in them are ripe. The presence of certain bacteria in the cultures greatly favors the formation and proper development of the perfect forms of species of Fusarium. A certain bacterium which was found in a contaminated culture when added to cultures of Fusarium having perfect forms favored the formation of perithecia so much that practically 100 per cent of the cultures to which this organism was added developed numerous normal perithecia, while even under best conditions only a small number of the cultures to which this bacterium was not added produced perithecia. What this organism is and whether other bacteria can produce the same result are not known.

Heretofore the whole work of producing the perfect stage of any ascomycete in pure culture has been handicapped to a certain extent by the fact that the cultures of such fungi dried out long before the formation and ripening of the perithecia. The addition of water to the cultures from time to time exposes them to contamination and varies the amount of moisture in the culture considerably. To avoid this the writer designed a special culture tube. This consists of a common test tube, to the lower end of which is attached a bulb (fig. 2). When the bulb is filled with water it will drain into the test tube as rapidly as the

water from the test tube evaporates or is used by the fungus. Such a tube provides stem or potato plug cultures with uniform moisture for four or five months without being refilled. This is as long a period of time as is necessary for the formation of perithecia in any case. When stems are used they can be placed directly in the test tube so as to reach the bottom, but when potato plugs, bean pods, or other cultural substrata are used it is better to place some cotton on the bottom of the test tube so that the plugs will be just above the water level. Such test tubes are handled in very much the same way as common test tubes, except that more care should be taken in sterilizing them, since a sudden decrease in the pressure in the sterilizer is likely to force the water out of the bulb into the tube.

PATHOGENICITY

PREVIOUS INVESTIGATIONS

A large number of Fusarium species have been reported by various workers as attacking the cereal crops in one way or another. In a large number of cases the particular organisms have been wrongly identified or not identified at all. The true relation of the various Fusarium species to the different diseases on the cereal crops attributed to these species is even less understood than their taxonomy. Indeed, there are but few papers out of over 200 references in which proof of the pathogenicity and true relation of some of these organisms to certain cereal diseases is given. No papers except those most directly connected with the problem can be mentioned here.

Selby (*9*) considered *Gibberella saubinetii* and its conidial form which he, following Saccardo, called *Fusarium roseum*, as the cause of the blighting of wheat heads, but he failed to produce the disease by inoculating heads with the conidia and ascospores of this organism. In 1909, Selby and Manns (*11*) succeeded in producing blighting of wheat and oat heads by spraying them during moist weather with a suspension of conidia obtained by washing samples of wheat, barley, oats, emmer, and spelt. In this way they thought they obtained the conidia of *F. roseum* and its perfect form, *G. saubinetii*. It is very likely that it was the conidia of *G. saubinetii* that caused blighting of the heads in their experiment, but it is incorrect to suppose that conidia of only this species of Fusarium are found on samples of cereals. They also showed that pure cultures of *G. saubinetii* from various sources when added to sterile soil in which wheat and oats were sown caused severe rotting of the roots and killing of the young seedlings.

Schaffnit (*8*), studying the cause of what is known as "snowmold" in Europe, showed that while *Fusarium nivale* Ces., the conidial form of *Nectria* (later *Colonectria*) *graminicola*, is the primary cause of "snowmold" of the cereal crops in Europe, the following organisms are also more or less responsible for this disease: *F. culmorum* (*F. rubiginosum*),

F. herbarum (*F. metachroum*) (13), *F. didymium*, *F. avenaceum* (*F. subulatum*), and *F. lolii*. He showed also that *F. nivale* causes not only the snowmold but also rotting of the roots and killing of the young cereal seedlings. Later it causes footrot of the grown plants, usually following the wounding of the plants by insects or other agencies. *F. nivale* attacks the heads of the cereals during the period beginning at blossoming time and extending to the ripening of the crops and causes blighting. In this connection he distinguished between primary infection, which takes place before the ripening of the plants, and secondary infection, during the period of maturity and harvest. In the secondary infection he found that not only *F. nivale* but also less parasitic Fusarium species play an important rôle.

Naumov (5), studying the cause of cereal headblighting, which is reported to be severe throughout Russia, found that *Gibberella saubinetii* and *Fusarium avenaceum* (*F. subulatum*) are the cause of this disease in Russia and Siberia, the first being common in the southern and the second in the northern part of the country.

Studying the pathogenicity of these organisms, Naumov reported that:

(1) Infection of the soil will result in the blighting of heads of wheat and barley. How the organisms introduced into the soil under sterile conditions reach the heads of the plants where they cause blighting is not quite clear. Throughout the paper Naumov states that the mycelium of these Fusarium species is found in all parts of the plants, but it is not very clear whether infection in the roots and the lower parts of the plant proceeds up the stem, becoming systemic, or whether the various parts are infected separately by external infections. Though this view is not directly and plainly stated, in many cases the reader will be led to believe that Naumov considers the infection systemic and that it proceeds from the roots up to the heads, since in many places in this paper he speaks of finding the mycelium of these organisms in all the tissues of roots, stems, heads, leaves, and sheaths, but nowhere causing any anatomical changes.

(2) Spores or conidia of the causal organisms when on the seed, or naturally infected seed, can cause blighting of the seedlings.

(3) Conidia, ascospores, and mycelium of the organisms, when placed on normal young plants, with or without wounding, cause infection.

(4) Spraying the heads of wheat, rye, and oats with a water suspension of conidia of these organisms produced typical blighting of the infected heads as observed in nature.

(5) The results given under (4) were also obtained with ascospores of *Gibberella saubinetii*.

(6) These organisms can invade the tissues of the seed, straw, and heads of the cereal crops after ripening and harvesting if conditions are favorable.

EXPERIMENTAL RESULTS

ISOLATIONS.—In the vicinity of Madison, Wis., where the writer secured most of his material, *Gibberella saubinetii* is the most common and most important cause of the headblight of the cereals, and the writer believes this to be true throughout the country. The following Fusarium species were also isolated from blighted heads and other parts of the cereal plants: *Fusarium avenaceum*, 10 times—4 times from wheat heads from a field near Madison and 6 times from a single sample of 10 blighted spelt heads from Hawthorne, which is located in the extreme northwestern part of Wisconsin; *F. herbarum*, 8 times—3 times from blighted wheat heads from a lodged wheat field near Madison, Wis., and 5 times from corn stalks; *F. culmorum*, once from a blighted wheat head from Arlington, Va.; *F. culmorum* var. *leteius*, twice from blighted wheat heads from a lodged wheat field near Madison, Wis.; *F. arcuosporum*, 10 times—once from a blighted oat seedling and 9 times from barley heads left in the field late in the fall and cornstalks early in the spring; *F. scirpi*, four times from blighted wheat heads from a lodged wheat field and once from a blighted wheat head from one of the Experiment Station plots at Madison, Wis., which was badly overgrown with weeds; *F. solani*, once from a grown wheat plant showing footrot; *F. arthrosporioides*, 5 times—once from a blighted wheat head from a lodged wheat field and 4 times from blighted barley heads; *F. redolens*, 3 times—once from a discolored rye stem near a node, once from a blighted wheat head from Knoxville, Tenn., and the third time from a blighted barley head from a weed-overgrown plot in the Experiment Station field, Madison, Wis.

On the other hand, *Gibberella saubinetii* was identified by the writer on over 2,000 blighted wheat, barley, rye, oat, and spelt heads from various parts of the following States: Wisconsin, Illinois, Minnesota, Indiana, Maryland, Kentucky, Ohio, Virginia, West Virginia, South Carolina, Georgia, Alabama, North Dakota, and Michigan. This shows that, from the standpoint of headblight of the cereal crops, *G. saubinetii* is the most important organism.

All species of Fusarium given here, including *Gibberella saubinetii*, were isolated originally by poured-plate dilution of conidia from distinctly blighted wheat heads. During the course of the work, however, some of these species were often isolated from blighted rye, barley, and oat heads, or stems, and from sheath, shank, root, and node rots of corn, or in a few cases from other hosts. The organisms attacking the cereal crops above the ground produce numerous conidia over the infected area. The conidia so produced are often normal and uniform in size and shape, and the trained student will not only have no difficulty in separating the various species before he has grown them under artificial conditions but he will be able also to determine in a general way the various species, at least the various sections to which they belong.

In order to prove that the Fusarium conidia produced on a blighted wheat head are the conidia of the causal organism and not of a secondary organism which has followed the first, parts of a large number of blighted wheat heads were washed in distilled water to moisten them and then disinfected by dipping them in 1 to 1,000 mercuric chlorid solution ($HgCl_2$) for two minutes. After this they were rinsed in distilled water and then transferred with a sterile needle to cooled poured plates of a suitable medium. In all cases only one organism was isolated from each blighted head, and this was in all cases the same as the one obtained from the conidia on this head. This is so true of the Fusarium organisms causing headblight that the causal organism upon a clean, undiscolored Fusarium-blighted head may almost surely, and even without microscopic examination, be described as one and pure. In rare cases the blighted heads may also be smutted, rusted, or brown spotted and discolored; and in such cases, of course, more than one organism may be found on a head. Such heads were discarded and never used for study or isolation.

Plain water agar [1] was used for diluting the conidia and for pouring the plates. After 12 to 24 hours the plates were examined microscopically, and single, germinating conidia were marked on the plate; then with a sterile needle made for the purpose they were transferred to test tubes containing suitable medium, usually hard oatmeal agar. In all cases five single, germinating conidia were transferred, with only one to each test tube. This was done to make sure that there was not more than one species of Fusarium present. Except in rare cases when some of the test tubes were contaminated during the manipulation with foreign organisms such as Penicillium or bacteria, all five test tubes yielded the same species. To make certain, however, that the cultures were free from bacteria they were transferred to plates, and second transfers were made from the margins of the plate colonies. The pure cultures so obtained were used as stock cultures for further study.

INOCULATION WORK.—In this paper only the results of inoculation with *Gibberella saubinetii* are given. The writer was able to produce blighting of heads of wheat and rye by inoculation with several of the species mentioned above and was able to produce more or less severe seedling-blight by inoculation with nearly all of them, but the conditions under which these species become pathogenic are not yet well understood.

SEED AND SOIL INOCULATION.—A number of methods have been used in artificially infesting soil with species of Fusarium. Most of them consist in growing the particular organism on a suitable medium and then introducing the whole culture into sterilized soil. Such a method is very good, except that it is an artificial one which does not reproduce

[1] One liter of distilled water and 25 gm. of bacto-agar.

the conditions that actually exist in nature. It introduces into the soil various substances, toxins perhaps, which may have some effect upon the final results. In order to avoid this and to make conditions in the greenhouse as natural as possible, only conidia were employed for inoculation of the soils used for testing the pathogenicity of *Gibberella saubinetii* on young seedlings. Practically all Fusarium species when grown under proper conditions will produce large masses of conidia, which can be gathered from the substratum with a flat needle, free from any conidiophores or mycelial hyphæ, and suspended in a test tube or flask of sterile distilled water. If the conidia are not abundant, a fairly heavy conidial suspension may be obtained by washing the culture with sterile distilled water and straining the water through sterile cheesecloth. Suspensions of conidia thus obtained were used for inoculating the seed by dipping the seed into it for a few minutes. Spore suspensions thus obtained were used for artificially infesting sterilized soil by pouring part of the suspension upon the soil in each of the pots and mixing it with the upper layer of soil. By this method only a comparatively small number of conidia and only a negligible amount of foreign matter were introduced into the soil.

In all the soil experiments the soil used was sterilized in pots in an autoclave for 1 hour at 15 pounds pressure. All the seed used for sowing was placed for several minutes in a weak solution of saponin [1] and shaken hard, the object being to moisten the seed thoroughly and to remove all air bubbles adhering to it. The seed was then soaked for 30 minutes in 1 to 1,000 mercuric chlorid solution. Seeds so treated proved to be perfectly sterile on the outside. However, the fungi present in their internal tissues are not affected by this treatment. For this reason, only seeds that were comparatively free from such fungi and healthy in appearance were used for experimental purposes.

Throughout the work 6-inch and 12-inch pots and garden soil were used for sowing the seed. In each case two pots were planted with infested soil or seed, and one pot was sown as a control. Each experiment was repeated several times.

Seed of wheat, rye, barley, and oats naturally or artificially infected with *Gibberella saubinetii*, or planted on sterile garden soil artificially infested with this organism, showed a decrease in germination. In the case of the seed naturally infected, the decrease in percentage of germination is greater and is variable, depending on the degree of infection and percentage of seed infected. This may vary from 2 or 3 per cent to as high as 50 per cent. Artificially infected seed or seed sown on infested soil also shows a lower percentage of germination than the controls similarly planted. Here, too, percentage of germination depends on the kind and condition of the seed. It may vary from 0 to as high as

[1] One hundred cc. of 50 per cent alcohol and 1 gm. of saponin.

15 per cent. Good, healthy, plump seed may show no decrease in germination, while weak and shriveled seed may show considerable decrease in germination.

Gibberella saubinetii, besides preventing some of the seeds from germinating, attacked from 10 to 40 per cent of the young seedlings, causing rotting and browning of their roots, bases, and sheaths (Pl. 3, A). A number of the plants so attacked, usually few under normal conditions, rot and die before reaching the surface of the soil. Others wilt and die after reaching the surface, while the large majority recover almost entirely and attain practically normal development. Over 20 spring-wheat plants which showed marked rotting and browning of the roots and bases caused by this organism while they were grown on sterilized soil from infected seed in pots out of doors, when transplanted to the pathological garden recovered rapidly and reached full development, producing heads as normal as those on the control plants. Only 2 of the plants so transplanted wilted shortly after the transplanting, and the writer is inclined to attribute the wilting more to the transplanting than to the parasitism of the organism. This fact shows that, although *G. saubinetii* when present on the seed will infect many of the seedlings, it is not able to injure them materially unless the plants are growing under extremely unfavorable conditions, as was the case with the plants shown in Plate 2, B. In this case, the experiment was conducted during February, 1918, at a time when there was a minimum of sunlight in the greenhouse and when all the greenhouse plants were consequently weakened. The results of the experiment are summarized in Table I.

TABLE I.—*Average results of two inoculation experiments on each of 2 wheat samples, sample 1 consisting of hand picked, healthy, plump kernels, and sample 2 consisting of hand-picked, healthy, but average kernels sown May 23, 1919, in pots kept out of doors*

Sample No.	Number of kernels.	Germination.	Number of healthy plants.	Number of plants showing rotting of roots and bases.	Number of killed plants.
		Per cent.			
1	Control, 100	91	89	2	0
	Inoculated, 100	90	75	15	2
2	Control, 100	76	71	5	0
	Inoculated, 100	69	42	27	6

While it was shown by numerous experiments that *Gibberella saubinetii* is able to decrease the percentage of germination of wheat, rye, barley, and oats and to cause rotting and browning of the roots and bases of some of the seedlings and even to cause wilting and dying of others, it was also noticed that this varied considerably from time to time and that some factors like light, temperature, moisture, and soil conditions have much to do with the degree and severity of infection.

Winter wheat, disinfected as described above, artificially inoculated with conidia of *Gibberella saubinetii*, and sown October 20, 1918, in five 12-inch pots of sterile soil with 10 kernels in each pot, was left in the greenhouse for 15 days and then taken out of doors, where it remained till July, 1919. A similar series of spring wheat similarly treated was sown on April 21 in pots of the same size but was left out of doors from the time of planting. Two pots sown with similarly treated but uninoculated seed were used as controls for each of the two series. In both series the plants recovered rapidly from the primary attack and grew normally, giving plants which were apparently normal, except that their bases and roots were slightly rotted and browned. With the coming of dry weather during the second half of June this rotting and browning of the roots and especially of the bases was intensified somewhat, and the plants began to wilt suddenly. In the field, wilting usually takes place at the time of heading or shortly after. The general symptoms accompanying wilting of fully developed plants are somewhat similar to those described for the footrot of the cereals in Europe and for "take-all" in Australia. *G. saubinetii* was isolated from the browned and rotted bases of the wilted plants in the foregoing experiments, as well as from those of some of the similarly wilted plants in the field.

HEAD INOCULATION.—While much work must be done before the nature and exact importance of the parasitism of *Gibberella saubinetii* on the underground portions of the cereal crops and the factors influencing or controlling it are fully understood, the question of headblighting due to this organism is much easier to follow and is, therefore, better understood.

The methods used in testing the pathogenicity of *Gibberella saubinetii* on wheat, rye, barley, oats, spelt, brome grass, quack grass, and timothy are very simple. They consist in producing a heavy suspension of conidia, either from heads already infected or from pure cultures, and spraying it by means of a small atomizer on a number of heads, usually 10, of the various hosts mentioned above when they are in the proper condition for infection. This method is successful when the weather is moist and cloudy. In dry weather this method will give either no results or only a very small percentage of infection. Certain results can be obtained only when the infected heads are in some way kept moist for at least three days after inoculation, and even this method will not give good results during extremely dry and hot weather. In the work described above the heads were kept moist by placing some moist absorbent cotton around the stems of a group of heads, then covering both the heads and the bundle of cotton around their stems with a glassine bag. The open end of the bag was tied around the stems just below the bundle of cotton. The heads so treated were heavy and required support. For this reason, garden stakes 5 or 6 feet tall were driven into the ground near the plants, and the bags covering the heads were tied loosely to them. The moist cotton inside of the bag kept the air comparatively moist and created

the condition desirable for successful infection. Since the glassine bags were transparent, the heads were not seriously deprived of sunlight. When the weather was very dry and warm the bags had to be opened and the cotton again moistened to saturation. All controls were treated in the same way as inoculated plants, except that they were sprayed with water to which no spores had been added.

Since *Gibberella saubinetii* usually produces very few conidia in culture, and since large quantities of spores were required for inoculations, it was necessary to contaminate the cultures purposely with a certain bacterium which has been found to bring about a great increase in sporulation. In this way large quantities of spores could always be obtained. The bacterium has not been identified, and the nature of its effect upon cultures of *G. saubinetii* is not known. Further study of this relationship is planned for the future.

The employment of such conidia for inoculation naturally raises the question whether the bacterium present has some effect on the pathogenicity of *Gibberella saubinetii* or whether it itself is pathogenic on wheat. In order to establish this, numerous wheat heads were inoculated at the same time with pure *G. saubinetti* conidia and others with a suspension of a pure culture of the unidentified bacterium. In all cases the heads inoculated with *G. saubinetii* conidia became blighted, while all heads inoculated with the bacterium suspension remained perfectly free from blighting or other injury. This shows that the bacterium favoring the sporulation and perithecia formation of *G. saubinetii*, as mentioned before, is not pathogenic on the wheat heads and has no effect upon the pathogenicity of *G. saubinetii*.

Wheat, spelt, rye, barley, and oat heads, as well as heads of *Agropyron repens* when inoculated with a conidial suspension or an ascospore suspension of *Gibberella saubinetii* became blighted. The blighting of *A. repens* proceeded exactly as observed in nature. In over 100 inoculation experiments in which over 3,000 heads of the various cereals, mostly wheat heads, were concerned, some infections always resulted. The number of blighted heads in each experiment varied from over 50 per cent to 100 per cent. In the majority of the experiments, all inoculated heads became infected and typically blighted. On many of these heads conidia were formed, and on some even the perithecia of *G. saubinetii* developed before the harvesting of the plants.

The inoculation experiments gave positive results from the time of blossoming till the latter part of the dough stage. Inoculation made before the first and after the second stage gave either negative or very doubtful results.

PERIOD OF INCUBATION

ON SEEDLINGS.—The period which elapses between the inoculation and the time the first symptoms of attack on the seedling roots appear varies so much that no definite incubation period can be given. It varies

considerably with the condition of the seed used. When light, shriveled seed is sown on infested soil, or when such seed is inoculated by being dipped in a suspension of conidia and then sown on sterile soil, the seedlings will succumb to the attack of the parasite much more rapidly than when healthy seed is used. Abundant watering of the plants also increases to some extent the rapidity of the attack.

In general, under greenhouse conditions, the first symptoms of root infection appear not earlier than the seventh day after sowing. Infection is usually abundant after the fourteenth day. When naturally infected seeds have been used on sterile soil the symptoms of root infection may appear even before the seventh day.

ON HEADS.—In head infection there is much less variation in the incubation period. In damp weather, the period that elapses between inoculation and the appearance of the first symptoms (water-soaking) varies from three to six days. In dry weather, symptoms of infection may not appear until after the first rain, or if heavy dew falls during the night and lasts for the greater part of the forenoon, symptoms of infection may appear from five to eight days later.

The rapidity with which the blight infection spreads from the point of infection to the rest of the head varies greatly. It varies considerably with different individuals and depends much upon the kind of weather. On healthy, vigorous, and more succulent plants the infection spreads much more rapidly than on plants of average vigor. Moist and cloudy weather, followed by warm and clear weather, greatly accelerates the rapidity of infection and killing, yet even under such conditions the infection may be restricted on many heads to a single spikelet, the rest of the head remaining healthy and developing perfectly normal, plump kernels.

For the study of the rapidity of the spread of the disease from the point of infection, heads showing primary infection were located daily and marked with tags so that they could be located again. Heads so tagged were examined every two or three days and the changes recorded. In this way the effect of the various factors affecting the rapidity of blight infection and killing were studied. The following are typical records of some infected heads, made in 1918:

N 1009, July 11, 1 spikelet infected. Infection at base of head.
July 14, 4 spikelets infected.
July 17, whole head killed.

N 101, July 11, 5 spikelets infected. Infection at middle.
July 14, 8 spikelets infected.
July 17, whole head killed.

N 1038, July 9, third spikelet from bottom infected.
July 14, 4 spikelets infected.
July 17, Whole head killed.

N 1039, July 9, 1 spikelet infected. Infection at middle.
July 14, 4 spikelets infected.

N 1039, July 17, 12 spikelets infected.
July 24, whole head killed.
N 1156, July 11, uppermost spikelet infected.
July 24, 1 spikelet infected. Plant almost ripe. Infected spikelet covered with Fusarium conidia.

There has been considerable discussion as to whether the headblighting of the cereal crops caused by *Gibberella saubinetii* and some other Fusarium species is the result of a systemic invasion of the host plants by these organisms. Naumov (5), as stated before, considers the invasion systemic. He finds the mycelium of the fungus in all parts of the plants and even in plants showing no blighting of the heads. He showed that infection of the heads can also take place externally.

Since there is uncertainty in determining from its appearance the kind and nature of any mycelium that may be present in the tissues of the cereal plants, it was thought that the easiest and only reliable way to show whether certain plants carry in their tissues the mycelium of *Gibberella saubinetii* or any other Fusarium species would be to plate out portions of such plants on some suitable artificial medium on which the organisms are known to thrive well. If they are present in the tissues of the plated plant they are sure to appear on the plates.

Wheat and rye plants with blighted heads where the infection from the heads has extended to the upper part of the upper internode, as previously described in this paper, were used for plating. Such peduncles were cut in portions 1 inch long, beginning from the end next to the blighted heads. These portions were disinfected on the outside by dipping them in 1 to 1,000 mercuric chlorid for two minutes. They were then rinsed in sterile distilled water and plated in order on hard potato agar. In all cases colonies of *Gibberella saubinetii* were formed over the portion next to the infected head and in some cases over the adjoining portion. The portion of the peduncle which was farthest from the head and perfectly green and free from discoloration never developed any fungous growth (Pl. 3, B). This shows very conclusively (1) that the infection on the cereal heads is local, and (2) that it proceeds from the head down and not from the roots up.

WEATHER CONDITIONS IN RELATION TO HEAD INFECTION

Weather is one of the important factors for the successful parasitism of *Gibberella saubinetii* and the various Fusarium species on the cereal crops. Indeed, it is the limiting factor for the occurrence of headblight under certain conditions, and its importance was noticed early by students of the subject. Dry weather with slight winds during and after the period of blossoming and extending well toward the dough stage will practically eliminate blight infection though all the other necessary conditions may be present. It was observed in many cases that in fields where there have been only few blighted heads before the

coming of rains and cloudy weather there was a marked increase in the number of blighted heads only a week after the rain. This was shown very plainly in experiment 22, one of the inoculation experiments in 1918.

At 7 o'clock in the afternoon, July 2, 1918, 60 wheat heads in one of the Wisconsin Experiment Station plots were sprayed with a suspension of *Gibberella saubinetii* ascospores and left uncovered.

On July 8, 1918, 12 heads, or 20 per cent, showed signs of first infection. Several days later there came a slight rain and the sky was cloudy for over a day. By the twentieth of the same month 28 heads, or 45 per cent, showed symptoms of blighting.

On the other hand, an experiment, which differed from the foregoing only in that the heads were kept moist artificially (see inoculation experiments, p. 25), showed 70 per cent infection on July 7, 1918. The number of the infected heads did not increase after the rainy and cloudy weather that followed. All controls in both experiments remained healthy. This case, which is one of several, shows that in the absence of proper weather conditions there is much less infection than when the weather is favorable. In experiment 20, in which the heads were kept moist, all the heads that were successfully infected showed infection within six days, and the coming of rain in this experiment did not increase the number of infected heads.

Not only does rainy and cloudy weather favor blight infection but it is also necessary for spore production, as already pointed out in this paper.

CULTURAL CONDITIONS IN RELATION TO HEADBLIGHT

Even though they were well developed and still apparently healthy and normal, the plants which were in shady places or overgrown by weeds were attacked by headblight and noderot to a much greater extent and by a greater number of the species of Fusarium than were plants which had a normal amount of sunlight. This was especially evident in one of the Wisconsin Experiment Station plots where a small area sown with barley and wheat was allowed to be overgrown by weeds. The blight infection on this plot was so abundant that in some small areas practically all the plants were infected. In general, the whole field had an average of 10 per cent of infection as compared with 5 per cent from neighboring clean fields. Another interesting fact was that nine different species of Fusarium, two of which have perfect stages, were isolated from blighted heads gathered from this small plot covering not over 200 square yards. *Gibberella saubinetii* was the most common and most destructive species.

Lodging of the fields also gives a marked increase of headblight infection. This was brought out especially well in a wheat field located two miles northeast of Madison, Wis., where the head infection among the

standing plants even in the worst-infected portions of the field never exceeded 15 per cent, while in the lodged portions of the field the head infection was, in some small areas, as high as 100 per cent. Considering that the field was not over two acres in extent, that the inoculum of *Gibberella saubinetii*, which was responsible for over 90 per cent of the infections in this field, was very uniformly distributed throughout the field, and that there were no other explanations for this great difference in degree of infection between the lodged and the standing plants, the effect of lodging on the prevalence of headblight infection becomes more striking.

VARIETIES IN RELATION TO THE DISEASE

During the summer of 1918 more than 30 varieties of wheat, both winter and spring, were grown by the Department of Agronomy, University of Wisconsin, on the University farm, and all were attacked more or less by headblight. There was marked difference between them in the degree of infection, but no variety was entirely free. As will be seen from the list given in Table II, among the varieties examined were representatives of types having very different morphological characters, from those which have very fine and succulent chaff to those which have hairy or very hard chaff.

Since the winter varieties examined were badly winter-killed, no significant count could be taken which would indicate their relative susceptibility to headblight. The spring varieties, on the other hand, were in very good condition and uniform throughout the series of plots.

The 15 spring-wheat varieties were sown in small plots of the same size, the plots being in one series which extended across the whole field. The whole series of varieties was repeated so that the variety planted on the first plot was repeated on the sixteenth plot, the variety planted on the second plot was repeated on the seventeenth plot, and so on. The plants in each plot were examined carefully and the blighted heads counted. The number of blighted heads of each variety in the two series was in many cases exactly the same. If there was a difference, it did not amount to more than two or three heads. The results are given in Table II.

These results, while not convincing, are very interesting, especially when we consider that all plots had the same preparation and cultivation, the same preceding crop, were on the same piece of land, that all varieties, while not in exactly the same stage of development, were in a stage in which they were susceptible to blight, and that the degree of infection of a certain variety was the same in the two series located a considerable distance apart.

One may suspect that the relative amount of infection of the seed used for sowing is the cause both of the difference of infection between different varieties and of the uniformity in degree of infection of the same

variety in both series. While this seems possible, it does not seem probable in this case. The plots were small and only 2 feet apart, so that if some plots were more heavily infected because of the more heavily infected seed sown on them the inoculum from them could easily have served for the plants in the neighboring plots only 2 feet away. The plot with the variety Preston × Kubanka cross (Wisconsin 101), which had 22 blighted heads, was between plots that had only 1 and 3 blighted heads, respectively.

TABLE II.—*Averages of actual counts of blighted wheat heads in two series of different varieties, arranged according to degree of infection*

Variety.	Wisconsin No.	Number of heads blighted.
Preston × Kubanka cross	101	22
Red Fife	46	20
Red Fife selection E. G. D. 9171	75	20
Marquis	50	15
Marquis selection	48	10
Pedigree Marquis	29	12
Red Fife selection	74	9
Fife, Minn. 163	Pedigree 34	9
Spring Velvet Chaff	60	7
Haynes Bluestem × Kubanka cross	102	7
Spring-wheat selection	76	3
Bluestem	Pedigree 35	3
Bluestem	Pedigree 36	1
Spring-wheat selection	98	1

The differences between varieties in susceptibility to blight was brought out more plainly in a field where two spring-wheat varieties, Marquis and durum, were sown side by side on the same piece of land, following corn. The infection of the Marquis wheat where the plants were standing was less than 1 per cent and from 10 to 15 per cent among the lodged plants, while the infection among the standing durum plants was from 9 to 10 per cent and as high as 100 per cent among the lodged plants.

Throughout the field there were numerous cornstalks with perithecia containing viable spores of *Gibberella saubinetii* and other parasitic species of Gibberella, as well as numerous viable conidia of several blight-causing Fusarium species. While we can doubt the result obtained with various varieties on the University plots, the results obtained on this field indicate clearly the existence of a difference in varietal susceptibility to head-blight. Further observations and experiments in this direction will, no doubt, be of great importance.

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A set of 100 plates prepared to accompany this work, but not published, is on file in the Office of Cotton and Truck Crop Disease Investigations of the United States Department of Agriculture.

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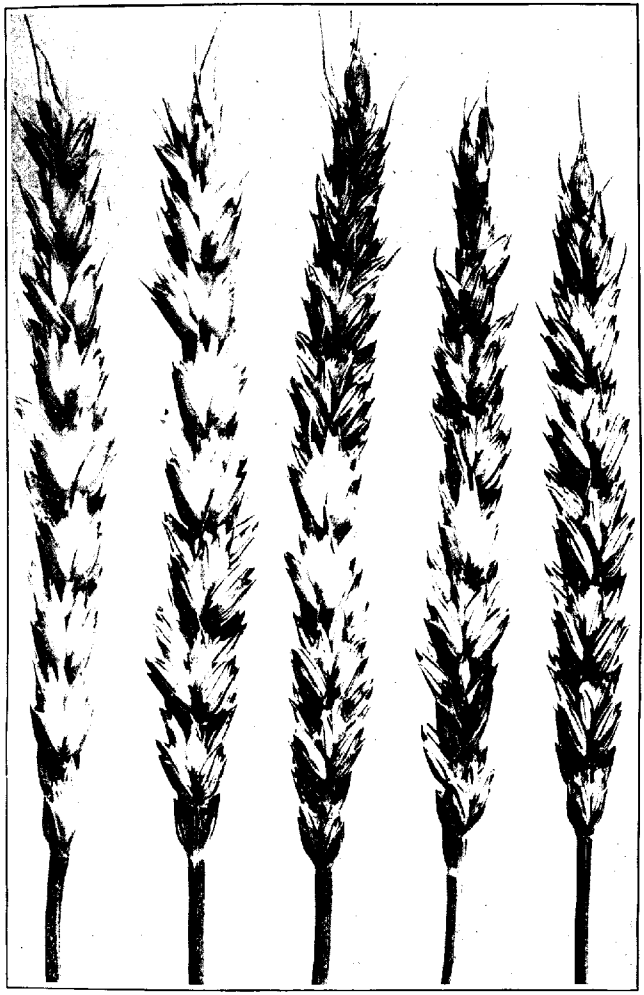
PLATE 1

Gibberella saubinetii:

Blighted ("scabbed") wheat heads. Control plant on the right of others, showing gradation of blighting to completely blighted head on the extreme left.



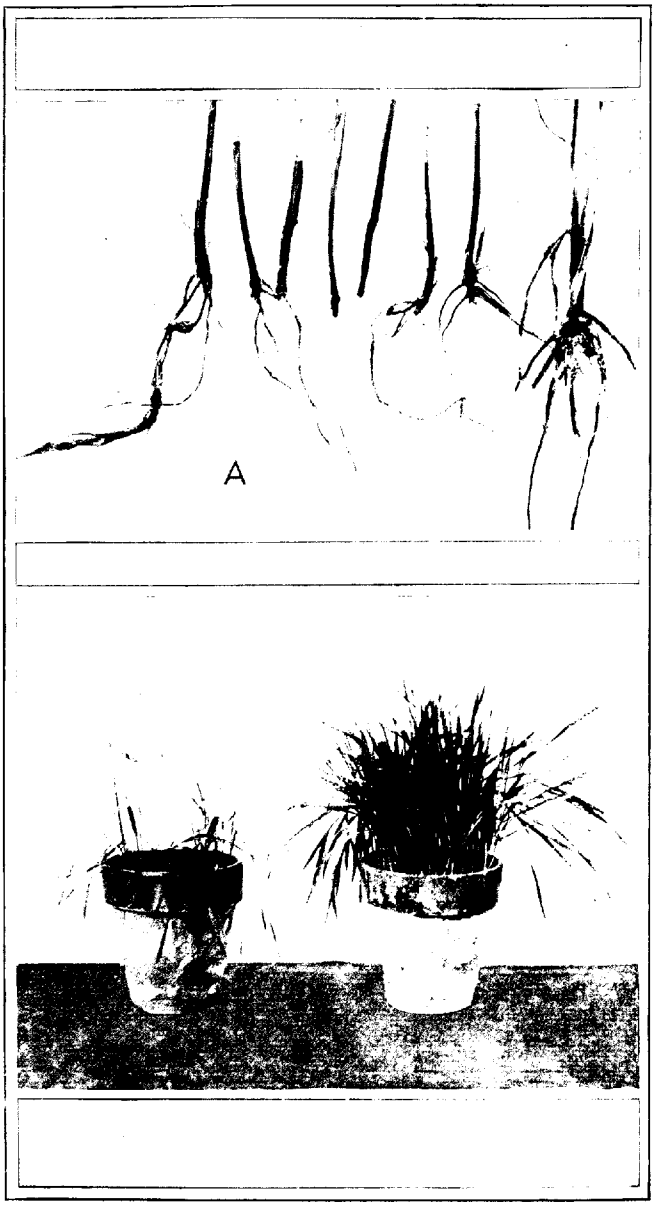

A

PLATE 2

Gibberella saubinetii:

A.—Footrot of wheat caused by Fusarium. The plants at the left were taken from soil which had been inoculated with *G. saubinetii*. The control plant at the right gives the comparative size of the normal wheat root system.

B.—Seedling-blight of wheat caused by *G. saubinetii*. The seed in the pot on the left was inoculated with *G. saubinetii* conidia before planting. The control pot on the left was planted with clean seed. Germination was reduced, and many of the seedlings were killed.

PLATE 3

A.—Fusarium seedling-blight. The normal plant is on the left. The other five show the gradations in blighting caused by *Gibberella saubinetii*.

B.—Tissue invaded by *G. saubinetii* in causing the headblight of wheat. Each group includes the four consecutive sections which, after surface sterilization, were cut from the upper internode of a wheat culm having a blighted head, the left segment in each group being the upper. These were then incubated on agar plates. Note that only the sections nearest the head were invaded by the fungus.

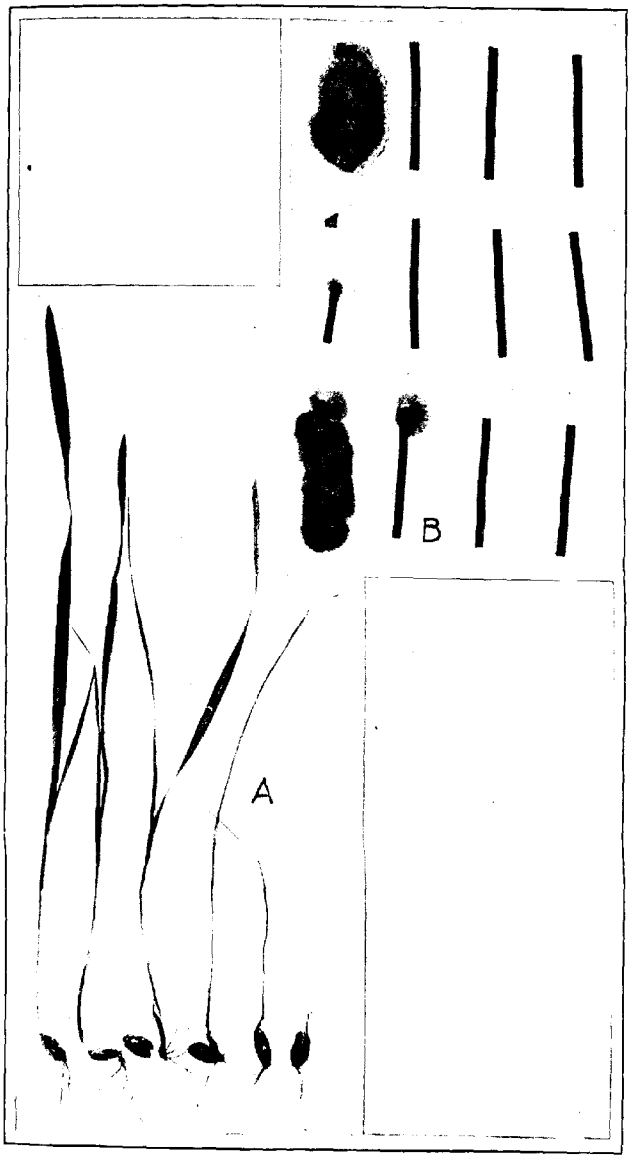

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PLATE 4

Gibberella saubinetii:

A.—Kernels blighted and shriveled by Fusarium-blight. Wheat kernels above are typical of Fusarium-blight. They are shriveled and much lighter than the normal kernels below.

B.—Perithecia development of *G. saubinetii* on an infected wheat head.

CAUSE OF LIME-INDUCED CHLOROSIS AND AVAILABILITY OF IRON IN THE SOIL

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CAUSE OF LIME-INDUCED CHLOROSIS

INTRODUCTION

Some years ago a study was made of a chlorosis of pineapples that occurred on certain soils in Porto Rico (*12*).[1] The particular type of chlorosis was confined to calcareous soils and seemed to be induced by a disturbance in the mineral nutrition of the plant. This disturbance appeared to consist in a lack of iron in the plant ash or in a diminished amount of iron combined with an increased amount of lime. Considerable work has since been carried on to determine more exactly the manner in which carbonate of lime in the soil induces chlorosis in the plant. The work comprises a number of direct experiments on the cause and cure of chlorosis as well as general studies in plant nutrition undertaken to gain information necessary for interpreting results obtained in the experiments on chlorosis. Since the more general work on plant nutrition has been published elsewhere, only the results will be referred to here.

In the following pages the more important facts already established concerning the cause of lime-induced chlorosis are given, together with a full report of certain experiments on this subject hitherto unpublished.

EVIDENCE THAT CARBONATE OF LIME MAY INDUCE CHLOROSIS

Evidence that carbonate of lime produces chlorosis in certain plants naturally falls into two classes, the results of soil surveys and the results of direct tests with natural or artificial calcareous soils. These two classes of evidence will be considered separately.

RESULTS OF SOIL SURVEYS

ECOLOGICAL STUDIES OF CALCIPHILOUS AND CALCIFUGOUS PLANTS.—Under the heading of soil surveys, reference should be made to the extensive literature on calciphilous and calcifugous plants. This literature, of which Roux (*39*) gives a complete bibliography up to 1900, consists chiefly of observations concerning the confinement of certain plants to calcareous or noncalcareous soils. While most of these

[1] Reference is made by number (italic) to "Literature cited," p. 59–61.

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Key No. B-16

observations do not deal directly with chlorosis, all are related to this subject, since calcifugous plants are often chlorotic on calcareous soils and since an exposition of the causes of chlorosis may afford an explanation of the calcifugous character of some plants.

There are a few plants which are very generally classed as calcifugous. Among these are the following: Maritime pine (*Pinus pinaster*) (*9*) chestnut (*Castanea vesca*), blueberry (Vaccinium), yellow and blue lupines (*Lupinus luteus* and *L. angustifolius*), certain species of sphagnum moss, etc. Cases have been recorded, however, where some plants generally considered calcifugous have been found growing on calcareous soils (*7*).

Probably the unsuitability of calcareous soils for certain plants is due not to carbonate of lime itself but to some soil characteristic usually associated with carbonate of lime. This being so, calcifugous plants might occur on certain calcareous soils provided some factor were operating to counteract the inhibiting characteristic usually associated with carbonate of lime.

STUDIES OF CHLOROTIC PLANTS.—Besides the soil surveys of calcifugous plants, there are several soil surveys which deal directly with the appearance of chlorosis in cultivated plants.

A case that has been the subject of much study is that of European grapes grafted on certain American stocks. When these were introduced on the calcareous soils of France and Germany they became chlorotic. Several soil surveys and many observations prove that the chlorosis is confined to calcareous soils and that there are varietal differences among grapes with respect to their resistance to lime (*22, 30, 33, 39 Viala and Ravaz, 45*). The accumulated data do not show, however, that all soils containing more than a certain percentage of carbonate of lime produce chlorosis in these varieties of grapes.

The chlorosis and failure of chestnut trees on most soils containing more than 3 per cent of carbonate of lime has been well established through soil surveys and through observations by Fliche and Grandeau (*10*), Piccioli (*36*), Vallot (*44*), and others. Vallot (*44, p. 202*) states that Dr. Bonnet reported that the chestnut failed to grow in a calcareous soil of Dijon, but when it was grafted on an oak it grew superbly.

That yellow and blue lupines and serradella become chlorotic when planted on calcareous soils is common knowledge in the calcareous districts of France and Germany, 2 per cent of carbonate of lime usually being sufficient to affect these plants.

A soil survey in Porto Rico showed that a chlorosis of pineapples was confined to the calcareous soils (*12, p. 8–18*). The only calcareous soils not producing chlorotic pineapples on which data could be obtained were some from the Florida Keys. These contained an exceptional amount of organic matter.

A chlorosis of sugar cane in Porto Rico was also found to be confined to

chlorosis. Green cane was found growing on a soil containing 76.70 per cent calcium carbonate (*19*).

Pears have frequently been reported as showing chlorosis on calcareous soils (*4*, *6*, *29*, *38*).

Instances have been noted where a great many other plants have become chlorotic on calcareous soils (*24*). Many of these cases are doubtless more or less exceptional, since some of the plants do not become chlorotic on most calcareous soils. Roux (*39*), without attempting a complete compilation, mentions some 50 genera and species of cultivated plants, ranging from mosses and orchids to maples and citrus trees, which have shown chlorosis when planted on soils containing calcium carbonate.

The results of the soil surveys and field observations seem to demonstrate conclusively that this type of chlorosis is confined under field conditions to calcareous soils. Probably no one species of plant, however, becomes chlorotic on all soils containing more than a certain percentage of calcium carbonate. Some plants are much more sensitive to carbonate of lime than others—that is, they become chlorotic on soils with lower lime contents and are less frequently found growing normally on limy soils.

The fact that plants very subject to chlorosis have been found in a few instances growing normally on markedly calcareous soils shows that the ability of calcareous soils to induce chlorosis does not depend entirely on the percentage of carbonate of lime in the soil. This fact also lends credence to the idea that it is not the carbonate of lime itself that induces chlorosis but some condition usually associated with the presence of carbonate of lime.

RESULTS OF VEGETATIVE EXPERIMENTS IN WHICH CHLOROSIS WAS PRODUCED BY NATURAL OR ARTIFICIAL CALCAREOUS SOILS

Compared with the mass of observations on the natural occurrence of chlorosis, there has been little reported in regard to inducing chlorosis by the use of calcium carbonate or in regard to direct tests of calcifugous plants in calcareous soils. There have been several vegetative experiments with yellow and blue lupines, however, where the addition of carbonate of lime to the soils caused a marked depression in growth and, in some cases at least, induced chlorosis. Concordant, positive results were secured by Heinrich (*23*), Meyer (*32*), Pfeiffer, and Blanck (*35*), the Agricultural Chemical Experiment Station at Breslau (*2*), Creydt (*5*), and Roux (*39*, *p. 147–185*).

Büsgen (*3*) grew the calcifugous broom (*Sarothamnus scoparius*), foxglove (*Digitalis purpurea*), and heather (*Calluna vulgaris*) in artificial calcareous and noncalcareous soils. The growth of all three plants was moderately to greatly depressed in the calcareous soil, although only broom was mentioned as showing chlorosis.

Roux (*39, p. 147*) grew some 20 species of calcifugous plants in calcareous soils. All species made diminished growth and became chlorotic in certain calcareous soils, while none showed chlorosis in the noncalcareous soil.

Piccioli (*36*) planted many varieties of chestnuts, together with Sarothamnus, Calluna, and Pteris, on soils with different additions of carbonate of lime. Most plants eventually died on the soil containing 12 per cent calcium carbonate.

Experiments at this Station showed that the mere addition of carbonate of lime to soils which normally produced green pineapples (*12, p. 20*) or rice plants (*13, p. 30*) caused the soils to produce chlorotic plants.

The preceding experiments seem to afford direct proof of the conclusions derived from field observations and from soil surveys that a chlorosis of some plants is caused by, or associated with, the presence of carbonate of lime in the soil.

MANNER IN WHICH CARBONATE OF LIME IN THE SOIL INDUCES CHLOROSIS IN THE PLANT

While it is quite generally conceded that carbonate of lime may induce a chlorosis in certain plants, there is a great diversity of ideas regarding the way the chlorosis is brought about. There are several classes of evidence or kinds of data on which conclusions concerning the nature of lime-induced chlorosis are based. These different kinds of evidence will be considered under the following heads: Evidence from analyses of plant ashes, effect of application of iron salts, effect of other lime compounds in inducing chlorosis, and effect of an alkaline reaction in inducing chlorosis.

RESULTS OF ASH ANALYSES OF PLANTS

In their work on the chlorosis of the chestnut and maritime pine Fliche and Grandeau (*9, 10*) analyzed leaves and branches of green and chlorotic trees. They concluded that the chlorosis and diminished growth of the trees on the calcareous soils were the result of an undue absorption of lime and a diminished absorption of other elements, notably potash and iron.

Schulze (*42*) analyzed the wood and leaves of green and chlorotic grapevines,[1] determining only lime, magnesia, potash, and soda. Compared with the green plants, the chlorotic ones had about one-half as much potash and soda and slightly more lime and magnesia in the ash.

Büsgen (*3*) analyzed the broom plants grown by him in calcareous and noncalcareous soils to determine lime and potash. The chlorotic and

[1] Analyses by Mach and Kurmann (*31*) are often quoted in this connection. The results probably have

green plants from the two soils had almost equal percentages of lime and potash in the ash, the percentage of total ash in the dry substance being higher in the chlorotic plants.

Numerous ash analyses were made at this Station from chlorotic and green pineapple plants grown in soils with and without carbonate of lime (*12*). Compared with the green plants, the chlorotic ones in the calcareous soils contained more lime and less iron in the ash; differences in other ash constituents were slight or inconstant, potash as a rule being fully as high in the chlorotic plants as in the green ones.

Green and chlorotic rice plants were also analyzed at different ages for their mineral constituents (*13*). In the case of rice grown 25 days, the chlorotic plants from the calcareous soils contained much more lime, less iron, and equal or greater percentages of potash in the ash than the green plants from the soil containing no carbonate of lime; but in the case of green and chlorotic rice of 84, 102, and 129 days' growth, the only constant difference in the ash of the two kinds of plants was a greater percentage of lime in the chlorotic plants. These analyses and a special study showed that the percentage of iron in the ash of rice diminished very markedly as the plants became more mature (*15*). Since plants affected with chlorosis matured much more slowly than normal plants, probably the iron contents of the 84-, 102-, and 129-day samples were affected more by the different maturities of the plants than by the character of the soils.

Four pairs of samples of green and chlorotic sugar-cane leaves were analyzed for their ash constituents. The leaves were selected from canes which were of the same size and age and which were growing on the same calcareous soil. In each case the chlorotic leaves had a distinctly lower percentage of iron in the ash than the corresponding green leaves (*19*).[1]

A summary of the evidence from ash analyses in regard to the cause of lime-induced chlorosis is as follows: Lime was determined in all seven species of plants analyzed by the different investigators, and in five cases it appeared that an excessive absorption of this element might be a cause of chlorosis; in two cases it appeared that it was not. Potash was determined in six of the different plants, and in only three cases did it appear that a lack of potash might be a cause of chlorosis. Iron was determined in five of the plants, and in all five cases it appeared that the chlorosis might be due to a deficiency of this element.

The weight of the evidence from the ash analyses seems to be that a deficiency of iron in the ash is at least one cause of the chlorosis and that possibly an excess of lime is also a cause. Against this conclusion there is the opinion of many physiologists, as Euler (*8*), Jost (*28*), and Sorauer

[1] In a fifth comparison, leaves of green, slightly chlorotic, and chlorotic cane were analyzed, the canes being of equal age but of markedly different size when grown on calcareous and noncalcareous soils. The chlorotic leaves contained very slightly more iron than the green leaves. In this case, it is believed that the maturities of the plants and the different ages of the leaves were the chief factors influencing the iron content (*19*, p. 15).

(*43*), that lime-induced chlorosis is caused chiefly by a lack of potash in the plant ash. This opinion is evidently based only on the analyses of Fliche and Grandeau (*9*, *10*, *11*) and on those of Schulze (*42*). If a lack of potash in the ash were the cause of the chlorosis, plants grown in non-calcareous soils and in water cultures, under controlled conditions, with an insufficient supply of potash, should show this type of chlorosis. In such cases, however, the lack of potash is indicated by the appearance of brown spots on the leaves and not by a yellowing.[1] However, it has not been shown that a combined excess of lime and deficiency of potash would not produce chlorosis.

The reliability of ash analyses as the sole means of diagnosing the cause of chlorosis is questionable. At the most, the results of ash analyses should be taken as merely indicating the cause or as confirming other evidence. The ash compositions of normal plants show such wide variations and are affected by so many conditions that it is sometimes unsafe to assume that of two lots of plants those which have made the better growth have an ash composition more nearly normal.

Aside from difficulties in properly interpreting the results of ash analyses, it is sometimes doubtful whether the samples selected for analysis are truly comparable, even when whole plants are taken. This uncertainty was demonstrated in the analyses of rice, previously referred to. The practice of taking only a portion of a plant for analysis is also susceptible to error, especially where iron is to be determined. Since iron appears to be relatively immobile in the plant after it is once transported to the leaves, certain leaves of a plant might contain a sufficiency of iron while other leaves and the plant as a whole might lack iron (*16*).

EFFECT OF APPLICATION OF IRON SALTS TO CHLOROTIC PLANTS

Eusebe Gris, in 1845 (*20*), and later Sachs (*41*) and other investigators (*12*, *21*, *25*, *26*, *27*) showed that various plants which became chlorotic on calcareous soils could be cured by applying ferrous sulphate to the leaves. This treatment and the improved one of Rassiguier (*37*), that of brushing cut surfaces of pruned vines with a concentrated solution of ferrous sulphate, have been rather generally used on grapevines which became chlorotic on the calcareous soils of France and Germany.

Various investigators have found that while iron salts were effective in overcoming chlorosis when applied to the stems and leaves of plants, they were ineffective when applied to the soil, even if used in considerable quantity. Sachs (*41*), however, observed that where the roots of plants were not completely surrounded by earth, as in the case of pot-bound plants, applications of ferrous sulphate to the soil did cure the chlorosis.

[1] If potash is concerned in the formation of starch from sugars, a low percentage of potash in chlorotic plants might be a secondary result of the chlorosis. With insufficient iron, chlorophyll formation is depressed, less sugar can be synthesized, and little potash would be needed.

Since ferrous sulphate is, of course, immediately transformed into ferric carbonate in a calcareous soil, it seems evident that calcium carbonate renders ferric carbonate unavailable, or less available, to certain plants.

It has been repeatedly demonstrated that the effectiveness of spraying with ferrous sulphate is due only to the iron and that only soluble iron salts are effective (*12*, *21*, *25*, *26*, *27*).

EXPERIMENT I.—The results in Table I show the effect of an iron spray upon chlorotic rice growing in a calcareous soil. The plants were grown in the open from February 29 to July 13, 1912, in small brick compartments with 36 plants to each compartment. Each compartment held about 200 pounds of heavy loam soil and received 5 gm. nitrogen, 3.4 gm. phosphoric acid, and 5 gm. potash, derived from various commercial fertilizers. The plants sprayed with ferrous sulphate were given 4 applications of a 0.5 per cent solution and 12 applications of a 1 per cent solution.

TABLE I.—*Effect of an iron spray upon chlorotic rice plants grown on calcareous soils*

Test No.	Calcium carbonate content of soil.	Treatment of plants.	Green weight of plants per compartment.		
			Series A.	Series B.	Average.
	Per cent.		*Gm.*	*Gm.*	*Gm.*
1	0	Unsprayed	1,022	1,071	1,047
2	0	Sprayed with ferrous sulphate	1,088	1,040	1,064
3	30	Unsprayed	4	(a)	
4	30	Sprayed with ferrous sulphate	946	894	920
5	50	Unsprayed	242	702	472
6	50	Sprayed with ferrous sulphate	874	893	884

[a] Some plants were eaten by mole cricket, but according to comparative growths of plants before any were eaten, the weight would have been about 250 gm.

Twenty-one days after planting, the plants in the calcareous soils were markedly chlorotic, and spraying was begun at that time. Seven days later, after nine sprayings, the sprayed plants in the calcareous soils were much superior to the unsprayed in color and growth. All plants in the noncalcareous soil had a good color at all times. (Pl. 5, A.)

The results obtained by treating the leaves and stems of chlorotic plants with iron salts show clearly that a lack of iron in the plant is at least one of the causes of lime-induced chlorosis. This conclusion is substantiated by the results of ash analyses of the plants. But this work does not show: (1) whether the lack of iron in the plant is due to a low availability of iron in the soil or to reactions in the plant rendering ineffective the iron absorbed; (2) whether an increased absorption of lime is a contributory cause of chlorosis; or (3) whether the reaction of the soil has any effect on the appearance of chlorosis, aside from affecting the iron supply.

EFFECT OF COMPOUNDS OF LIME IN INDUCING CHLOROSIS

To see whether lime salts in general induce chlorosis in certain plants, experiments have been conducted with calcium carbonate, sulphate, phosphate, and silicate. The effects of these compounds on the growth of lupines have been determined by Heinrich (*23*), Pfeiffer and Blanck (*35*), and Creydt (*5*). The calcium sulphate did not induce chlorosis but depressed growth considerably, although much less than the calcium carbonate, while calcium phosphate and silicate were markedly toxic. The toxicities of the latter two substances were attributed to their acid and alkaline reactions, respectively.

Large quantities of gypsum depressed the growth of pineapples about 20 per cent but did not cause chlorosis (*12*). Various experiments were conducted to determine the effect on rice of large amounts of assimilable lime in the form of gypsum.

EXPERIMENT II.—In this experiment, rice plants were grown from December 17, 1912, to May 20, 1913, in small brick compartments, with 24 plants to each compartment. Each compartment held about 200 pounds of soil fertilized with 30 gm. sulphate of ammonia, 20 gm. nitrate of soda, 30 gm. acid phosphate, and 18 gm. muriate of potash added in two applications. The results are shown in Table II.

TABLE II.—*Effect on the growth of rice of adding gypsum to the soil*

Test No.	Kind of soil.	Gypsum ($CaSO_4 \cdot 2H_2O$) added.	Green weight of plants per compartment.			
			Series A.	Series B.	Series C.	Average.
		Per cent.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	Loam	0	1,218	1,229	1,452	1,300
2	do	15	312	446	382	380
3	Clay	0	.....	808	840	824
4	do	15	936	958	842	912

During the first four weeks the plants were all of good color, but later the plants in the loam soil containing gypsum became yellow, though not typically chlorotic.

EXPERIMENT III.—A second experiment was conducted to see whether large amounts of gypsum would depress the growth of rice if the plants were sprayed with ferrous sulphate. The compartments contained about 200 pounds of a sandy soil and received 45 gm. sulphate of ammonia, 30 gm. acid phosphate, and 18 gm. muriate of potash. In each compartment 22 plants were grown. The plants treated with ferrous sulphate were sprayed twice with a 0.1 per cent solution, five times with a 0.15 per cent solution, once with a 0.2 per cent solution, three times with a 0.75 per cent solution, and seven times with a 1 per cent solution. The results are given in Table III.

TABLE III.—*Influence of spraying with ferrous sulphate on the depressing effect of gypsum*

Test No.	Gypsum ($CaSO_4 \cdot 2H_2O$) added.	Treatment of plants.	Air-dried weight of plants per compartment.			
			Series A.	Series B.	Series C.	Average.
	Per cent.		*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	0	Unsprayed	521	503	475	500
2	0	Sprayed with ferrous sulphate.	478	525	525	509
3	15	Unsprayed	324	300	366	330
4	15	Sprayed with ferrous sulphate.	395	364	372	377

The plants in the soil to which gypsum had been added were markedly behind the others in growth from the start, and they were at times of poorer color, though they were never typically chlorotic. Plants in soil without gypsum were of good color at all times. No effect from spraying with ferrous sulphate was observable.

EXPERIMENT IV.—A further experiment with gypsum and ferrous sulphate was conducted in pots in a glass house. Six rice plants per pot were grown from July 7 to October 25, 1913. Each pot contained 37 pounds of sandy soil, to which 13 gm. ammonium sulphate, 11 gm. acid potassium phosphate, and 3.6 gm. sulphate of potash were applied. The moisture content of the soil was maintained at the optimum. The results appear in Table IV.

TABLE IV.—*Influence of different treatments with ferrous sulphate on the depressing effect of gypsum*

Test No.	Gypsum ($CaSO_4 \cdot 2H_2O$) added.	Treatment of plants.	Air-dried weight of plants per pot.				
			Series A.	Series B.	Series C.	Series D.	Average.
	Per cent.		*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	0	.....	114	113	113	118	115
2	15	.....	85	92	68	67	78
3	15	Ferrous sulphate, 2.2 gm., added to soil.	.....	50	86	85	74
4	15	Plants sprayed eight times with 1 per cent solution of ferrous sulphate.	67	60	60	78	66

The color of the plants grown in the soil to which gypsum was added was as good as that of the controls up to the eighty-fifth day, but from the eighty-fifth to the one hundred and tenth day the former were yellow. The controls were always of a good green color. No effect from either of the treatments with ferrous sulphate was observable.

SUMMARY.—In all the tests, except that with the clay soil, calcium sulphate depressed the growth of rice and induced a certain amount of yellowing. The yellowing, however, was not that typical of lime-induced

chlorosis. Spraying with ferrous sulphate and adding ferrous sulphate to the soil failed to increase the growth or improve the color of plants growing in the soil containing calcium sulphate. That calcium sulphate increased the amount of lime in the plants may be seen by the analyses in Table V of plants 65 days old from experiment II.

TABLE V.—*Ash analyses of plants from experiment II*

Test No.	Kind of soil.	Gypsum ($CaSO_4 \cdot 2H_2O$) added.	Carbon-free ash in dry substance of plants.	Analyses of carbon-free ash.							
				Silica (SiO_2).	Lime (CaO).	Magnesia (MgO).	Potash (K_2O).	Soda (Na_2O).	Iron (Fe_2O_3).	Phosphoric acid (P_2O_5).	Sulphur (SO_3).
		P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
1	Loam..	0	16.75	54.70	3.63	4.20	24.77	5.48	0.55	2.58	2.19
2	...do...	15	13.92	47.24	6.45	5.22	26.81	4.54	.53	4.01	6.71
3	Clay...	0	14.14	50.77	3.87	4.80	25.11	4.65	.62	2.31	2.34
4	...do...	15	13.08	45.04	6.40	4.77	28.34	7.00	.38	3.13	4.76

It will be noted that the calcium sulphate increased the percentages of lime and sulphur in the plant ash and diminished the percentage of silicia but had little effect on the other constituents.

The injurious effect of calcium sulphate on rice might have been due to several causes. A large amount of gypsum evidently maintains a solution more concentrated than that existing in any except alkali soils. There is also the possibility of hydrogen sulphid being formed by bacteria reducing sulphates. This occurred with soil preserved in a sample jar, although such a result was not to be expected in what appeared to be a normally aerated soil. The fact that calcium sulphate did not depress growth in the clay soil lends credence to the view that the injurious effect might have been that of a too concentrated soil solution.

In order to make sure that an increased assimilation of lime is not a cause of chlorosis, a test was conducted with lime salts applied to the leaves. The results, given in experiments V and VI, to be described further on, seemed to show definitely that an increased assimilation of lime does not induce chlorosis.

Although excessive quantities of various lime compounds seem to be more or less injurious, each one appears to act differently; there is no evidence of a general "lime effect" in inducing chlorosis.

EFFECT OF AN ALKALINE REACTION IN INDUCING CHLOROSIS

Pfeiffer and Blanck (*35*) in their first work on the intolerance of lupines for calcareous soils concluded that lupines are especially sensitive to an alkaline reaction and that the carbonate of lime not only depresses the absorption of nutrients but is directly injurious to the roots of the plants. While the alkaline reaction of carbonate of lime is evidently a factor in the chlorosis, it is very evidently not directly injurious to roots of even

rice at this Station that the ratio of root to top growth was much increased in calcareous soils and solutions (*12*, *17*). The stimulating effect of carbonate of lime on the root growth of plants which are not calcifugous has been frequently noted.

In work with pineapples it was shown that the alkalinity induced by increasing amounts of carbonate of soda greatly depressed growth without affecting the color of the plants (*12*, *p. 31*).

Work with rice in water cultures seemed to show definitely that the alkalinity of carbonate of lime is not directly injurious to this calcifugous plant, nor is the alkalinity in itself the cause of chlorosis (*17*). Rice was grown with different quantities of iron from different sources in nutrient solutions which were acid, neutral, and alkaline from carbonate of lime. A summary of the relative growths made under the different conditions is given in Table VI.

TABLE VI.—*Relative growths of rice plants with different amounts of iron from various sources in acid, neutral, and alkaline solutions*

Source of iron in nutrient solutions.	Iron per liter added to nutrient solutions.	Relative growths in—		
		Acid solution.	Neutral solution.	Alkaline solution.
	Gm.			
Ferrous sulphate	0.002	100	88	.....
Do	.....	100	74	51
Do	.....	100	95	95
Do	.008	100	105	.....
Do	.004			
Do	.002	100	107	.....
Do	.008	100	132	.....
Do	.....	100	111	2
Ferric chlorid	.002	100	99	26
Do	.008	100	107	26
Ferric citrate	.002	100	85	86
Do	.....	100	94	97
Do	.....	100	101	104
Do	.008	100	85	58
Ferric tartrate	.002	100	80	76
Do	.008	100	96	100
Dialyzed iron	.008	100	27	.....

Where growth was depressed to any extent the plants were more or less chlorotic, and that this chlorosis was evidently due to lack of iron was shown by analyses of the plants and by treatment of the leaves with ferrous sulphate. The work showed quite definitely that rice is not particularly sensitive to the reaction of carbonate of lime, except as the reaction influences the availability of the iron. When the proper form of iron was used in the proper quantity, the growth and appearance of the plants were as good in the solutions containing carbonate of lime as in the acid or neutral solutions.

The preceding results seem to show that neither increased assimilation of lime nor mere alkalinity causes chlorosis. It remained to be seen

whether the combination of the two conditions would induce a typical chlorosis. It was thought that this might be determined experimentally by growing rice on soil to which sodium bicarbonate had been added to render it alkaline and then inducing an increased assimilation of lime by spraying the plants with calcium chlorid and gypsum. In case these treatments should induce a chlorosis identical with that produced by carbonate of lime, spraying with ferrous sulphate should cure it. Accordingly, some plants grown in the soil with sodium bicarbonate were sprayed with lime salts alone, with ferrous sulphate alone, and with both lime and iron salts. Plants grown in a soil without sodium bicarbonate were also sprayed as described above in order to check the results.

The experiment was carried out twice, once in the open, using small brick compartments, and once in the glass house, using pots. Results are given under the heads of experiments V and VI. The sodium bicarbonate was added in several doses until it became evident that sufficient had been applied to affect growth. More was required for the soil in the open than for that in the glass house, since the former was exposed to leaching.

EXPERIMENT V.—This test was run from November 8, 1913, to January 20, 1914. Each plot containing 150 pounds of sandy soil received 45 gm. sulphate of ammonia, 30 gm. acid phosphate, and 18 gm. muriate of potash. Thirty rice plants were grown on each plot. The results are given in Table VII.

TABLE VII.—*Effect of sodium bicarbonate, lime, and iron on the growth of rice: Experiment V*

Test No.	Approximate percentage of sodium bicarbonate in soil.	Treatment.	Air-dried weight of plants per plot.		
			Series A.	Series B.	Average.
			Gm.	*Gm.*	*Gm.*
1	0	None	144	156	150
2	0	Sprayed 31 times with 0.5 to 2 per cent solutions of calcium chlorid and gypsum	142	152	147
3	0	Sprayed 31 times with 0.5 to 2 per cent solutions of calcium chlorid and gypsum and 8 times with 0.5 to 1 per cent solutions of ferrous sulphate	163	166	165
4	0.2	None	100	104	102
5	.2	Sprayed 31 times with 0.5 to 2 per cent solutions of calcium chlorid and gypsum	101	82	92
6	.2	Sprayed 31 times with 0.5 to 2 per cent solutions of calcium chlorid and gypsum and 8 times with 0.5 to 1 per cent solutions of ferrous sulphate	110	100	105
7	.2	Sprayed 8 times with 0.5 to 1 per cent solutions of ferrous sulphate	.....	113	113

The plants in the soils containing sodium bicarbonate became somewhat yellow, though the yellowing was not that of typical lime-induced chlorosis. The yellowing, however, was not increased by the lime spray, nor was it overcome by the iron spray. The lime and iron sprays also had no effect on the appearance of the plants growing in the soil containing no sodium bicarbonate.

EXPERIMENT VI.—In this test, conducted from November 4, 1913, to March 12, 1914, 7 rice plants were grown per pot. Each pot contained 35 pounds of sandy soil and received 6 gm. ammonium nitrate, 1.3 gm. potassium acid phosphate, and 2.5 gm. potassium sulphate. The moisture content was maintained at 25 per cent of the dry weight of the soil. The results are shown in Table VIII.

TABLE VIII.—*Effect of sodium bicarbonate, lime, and iron on the growth of rice: Experiment VI*

Test No.	Approximate percentage of sodium bicarbonate in soil.	Treatment.	Air-dried weight of plants per pot.		
			Series A.	Series B.	Average.
			Gm.	*Gm.*	*Gm.*
1	0	None	83	71	77
2	0	Plants sprayed 23 times with 0.5 to 2 per cent solutions of calcium chlorid and sulphate	68	72	70
3	0	Plants sprayed 23 times with 0.5 to 2 per cent solutions of calcium chlorid and sulphate and 7 times with 0.5 to 1 per cent solutions of ferrous sulphate	65	70	68
4	0.08	None	44	56	50
5	.08	Plants sprayed 23 times with 0.5 to 2 per cent solutions of calcium chlorid and sulphate	52	51	50
6	.08	Plants sprayed 23 times with 0.5 to 2 per cent solutions of calcium chlorid and sulphate and 7 times with 0.5 to 1 per cent solutions of ferrous sulphate	42	39	40
7	.08	Plants sprayed 7 times with 0.5 to 1 per cent solutions of ferrous sulphate	49	60	55

The appearance of the plants in this test was the same as in experiment V.

The plants from experiment V were analyzed for their ash constituents, and the results appear in Table IX. The plants were washed immediately after cutting, so no lime salts remained on the leaves. While it is believed that all iron applied as a spray was also removed by washing, it is possible that some iron in the form of ferric oxid might have remained adhering to the leaves; hence, in the case of the plants sprayed with ferrous sulphate, it is possible that the analytical results may show more iron than was actually present in the plants.

TABLE IX.—*Analyses of rice plants growing in a soil with sodium bicarbonate and sprayed with lime and iron salts*

CONSTITUENTS OF ASH IN TOTAL DRY MATTER

Test No.	Approximate percentage of sodium bicarbonate in the soil.	Treatment.	Carbon-free ash.	Silica (SiO_2).	Lime (CaO).	Magnesia (MgO).	Potash (K_2O).	Soda (Na_2O).	Iron (Fe_2O_3).	Phosphoric acid (P_2O_5).	Nitrogen (N).
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	0	None	11.17	4.51	0.48	0.59	3.75	0.30	0.015	0.72	2.57
2	0	Plants sprayed with 0.5 to 2 per cent solutions of calcium chlorid and sulphate	14.12	6.83	.83	.64	4.31	.35	.021	.62	2.57
3	0	Plants sprayed with 0.5 to 2 per cent solutions of calcium chlorid and sulphate and 0.5 to 1 per cent solutions of ferrous sulphate	12.80	5.88	.71	.39	3.30	.47	.065	.69	2.74
4	0.2	None	14.98	8.06	.54	.82	3.44	1.02	.018	.69	2.80
5	.2	Plants sprayed with 0.5 to 2 per cent solutions of calcium chlorid and sulphate	15.56	7.84	1.10	.70	3.44	.88	.019	.66	2.91
6	.2	Plants sprayed with 0.5 to 2 per cent solutions of calcium chlorid and sulphate and 0.5 to 1 per cent solutions of ferrous sulphate	14.96	7.78	.81	.66	3.43	.73	.084	.68	2.73
7	.2	Plants sprayed with 0.5 to 1 per cent solutions of ferrous sulphate	12.41	5.98	.51	.68	3.20	.91	.063	.64	.....

CONSTITUENTS OF CARBON-FREE ASH

1	0	None	.....	40.42	4.29	5.27	33.61	2.72	0.14	6.44	.....
2	0	Plants sprayed with 0.5 to 2 per cent solutions of calcium chlorid and sulphate	.....	48.46	5.87	4.50	30.48	2.45	.15	4.39	.....
3	0	Plants sprayed with 0.5 to 2 per cent solutions of calcium chlorid and sulphate and 0.5 to 1 per cent solutions of ferrous sulphate	.....	46.01	5.53	3.04	29.72	3.70	.51	5.41	.....
4	0.2	None	.....	53.90	3.60	5.46	22.95	6.80	.12	4.59	.....
5	.2	Plants sprayed with 0.5 to 2 per cent solutions of calcium chlorid and sulphate	.....	50.34	7.07	4.52	22.18	5.65	.12	4.26	.....
6	.2	Plants sprayed with 0.5 to 2 per cent solutions of calcium chlorid and sulphate and 0.5 to 1 per cent solutions of ferrous sulphate	.....	52.01	5.74	4.44	22.96	4.91	.56	4.54	.....
7	.2	Plants sprayed with 0.5 to 1 per cent solutions of ferrous sulphate	.....	48.13	4.10	5.46	25.77	7.30	.51	5.18	.....

SUMMARY.—None of the sprays affected the growth or color of the plants, either in the normal soil or in the soil containing sodium bicarbonate. The amount of sodium bicarbonate required to depress growth was rather surprising, and from this fact it was suspected that the availability of iron was not noticeably depressed by sodium bicarbonate, at least not below the critical point. This was confirmed by the analyses of the plants and by the fact that spraying with ferrous sulphate effected no improvement in either the growth or color of the plants planted in the soil containing sodium bicarbonate.

The spraying with lime salts, however, notably increased the amount of lime in the plants without affecting the quantity of iron, and spraying with both lime and iron solutions increased the quantities of both elements in the plant. The yellowing and depression in growth produced by the sodium bicarbonate were probably due to an injurious degree of alkalinity, which must have been far greater than that which is produced by carbonate of lime.

The results of these experiments, where a large amount of sodium bicarbonate was required to depress growth, seem to show that the slight alkalinity of carbonate of lime could not be directly injurious to rice, nor could alkalinity in itself be the cause of chlorosis. While this experiment failed to yield the decisive answer expected, it is felt that the results point strongly to the conclusion that an increased assimilation of lime is not the cause of chlorosis.

CHLOROSIS DUE SIMPLY TO A DEPRESSION IN AVAILABILITY OF IRON IN THE SOIL

An attempt was made to demonstrate directly that the only action of carbonate of lime in inducing chlorosis lies in depressing the availability of the iron. It was thought that this demonstration could be accomplished by growing rice plants with their roots divided between two kinds of soil, one to contain carbonate of lime and all the mineral nutrients except iron, and the other to contain only iron. The attempt was not completely successful, due partly to a principle discovered later and partly to difficulties in execution. The principle which tended to make the results less striking than had been anticipated is the following: Plants apparently are unable to attain a maximum absorption of any one element with only a part of their roots (*18*).

Wire sieves were made which fitted into the tops of buckets. The buckets were filled with soil to within 1 inch of the bottom of the sieves, and the sieves were filled with about 2 inches of soil (Pl. 5, *B*). In this way an air space was left between the soil in the sieve and that in the bucket; this prevented any soil solution passing by capillary attraction from the soil below to that above. It was the intention at first to fill all except the control buckets with a calcareous soil containing all the nitrogen, phosphoric acid, and potash, and to fill most sieves with pure silica sand containing only iron. In conducting the experiment,

however, it was found necessary to apply a small amount of nitrogen, phosphoric acid, and potash to the sand in the sieve in order that the plants might develop sufficiently for their roots to penetrate the soil below.

The intention was that the plants should absorb practically all their nutrients from the soil in the buckets (a calcareous soil except in control buckets 1 to 4), but that in some cases the plants should be able to absorb iron from a lime-free medium in the sieve. If carbonate of lime affected the plants in any way except through depressing the absorption of iron, all plants should make equally poor growth; but if, on the other hand, the only action of the carbonate of lime lay in decreasing the availability of the iron, those plants that could draw iron from a medium containing no carbonate of lime should do much better than the others.

A preliminary test was run with two pots, No. 1 containing silica sand in the sieve and a calcareous soil in the bucket, and No. 2 containing silica sand plus carbonate of lime in the sieve and the same calcareous soil in the bucket as No. 1. Four gm. of ferrous sulphate were applied to both sieves (Pl. 6, A). The yields from pots No. 1 and 2 were respectively 169 gm. and 97 gm. of air-dried plants, the plants in No. 1 being green in color and those in No. 2 chlorotic.

EXPERIMENT VII.—The results of a more extended test are given in Table X. The plants were grown from October 22, 1912, to March 3, 1913. A large number of seeds were planted, but the plants in each pot were thinned to eight. The sieve of each pot contained 10 pounds of silica sand to which were added 0.45 gm. ammonium nitrate, 0.1 gm. acid potassium phosphate, and 0.2 gm. potassium sulphate. The bucket of each pot contained 23 pounds of soil and received 12 gm. ammonium nitrate, 3 gm. acid potassium phosphate, and 5.5 gm. potassium sulphate, in two applications. The moisture content of the soil was maintained at 31 per cent of the dry weight.

TABLE X.—*Effect of carbonate of lime in the soil on the availability of iron*

Pot No.	Treatment of soil in bucket.	Treatment of sand in sieve.	Green weight of plants per pot.				
			Series A.	Series B.	Series C.	Series D.	Average.
			Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1 to 4	None	None	206	171	204	.....	194
5 to 8	Calcium carbonate, 15 per cent.	None	137	224	156	161	170
9 to 12	...do	Eight gm. ferrous sulphate in four applications.	204	174	236	187	200
13 to 16	.....do	Eight gm. ferrous sulphate in four applications; 15 per cent calcium carbonate.	112	97	115	84	102

At 15 days after sowing the seed all plants were chlorotic except those in pots 13 to 16, and many died because of their inability to establish roots in the soil in the bucket. At 121 days the plants of pots 1 to 4 and 9 to 12 were green, while those of No. 5 to 8 and 13 to 16 were strongly chlorotic.

The plants encountered some difficulty in establishing their roots in the soil in the buckets; the roots after passing through the sieve often grew for a time on the surface of the soil. This retarded growth considerably, but when the roots once penetrated the soil, growth became normal. At the end of the experiment the greater part of the roots were in the soil in the bucket, where practically all the fertilizer was located.

The final yields of the plants and the chlorotic appearance of certain plants during the latter stages of growth confirm the idea that the only effect of carbonate of lime in inducing chlorosis lies in depressing the availability of iron. The plants in pots No. 9 to 12 and those in No. 13 to 16 were exposed to the same conditions except that the plants in No. 9 to 12 were able to draw part of their iron from a medium containing no carbonate of lime; this difference was sufficient to double the growth of plants. The plants of No. 9 to 12 had to assimilate practically all their mineral nutrients, except iron, from the same calcareous soil as the plants of No. 13 to 16; hence, if the carbonate of lime induced chlorosis by depressing the availability of any nutrients other than iron, or if an increased assimilation of lime were a contributory cause of chlorosis, the yield from pots No. 9 to 12 should have been practically the same as from No. 13 to 16.

The only apparent contradiction in this demonstration of the cause of lime-induced chlorosis lies in the fact pots No. 5 to 8 yielded more than No. 13 to 16. Plants in pots No. 5 to 8 evidently secured less iron than those in No. 9 to 12, for they made less growth; but if the sand in the sieve had been really iron-free they should have made no more growth than plants No. 13 to 16. Later work showed that, although no iron was added to the sieves of No. 5 to 8, doubtless the silica sand contained enough iron to cause the unanticipated growth. In work with nutrient solutions it was found that rice practically satisfied its iron requirements in a solution containing no more than 1 part of truly soluble iron in 10,000,000 parts of solution (*17, p. 5*).

On repeating this experiment the same difficulties were encountered, but the relative growths made by the differently treated plants were similar to those in the preceding test.

AVAILABILITY OF IRON IN THE SOIL

INTRODUCTION

Since the preceding summary of facts and experiments seems to indicate that lime-induced chlorosis is simply the result of insufficient available iron in the soil, evidently a knowledge of conditions affecting the

availability of iron in the soil is essential to a complete understanding of this chlorosis. If all the conditions affecting the amount of available iron in the soil were known, it would doubtless be possible to explain why some calcareous soils induce chlorosis when others do not; why in a sandy soil a smaller percentage of carbonate of lime is required to induce chlorosis than in a clay soil; why a calcareous soil that produces chlorotic plants at one time may not at another; and many other perplexing facts.

Since a method for determining the amount of available potash or phosphoric acid in the soil is still unknown, in spite of years of work, the prospect is not bright for even roughly determining the available iron by direct means; and to determine directly significant differences in amounts of available iron seems hopeless when plants obtain their iron from such exceedingly dilute solutions.

Soils which yield sufficient iron for the growth of plants may not show a detectable amount of iron in the water extract. In some cases the water extract of soils may show considerable iron, but the iron may be in a colloidal state and not in true solution. Colloidal iron was found unavailable for rice in water culture (*14*).

While there are great difficulties in the way of determining the small, significant quantities of soluble or available iron in the soil, it seems from the work of Morse and Curry (*34*), Ruprecht (*40*), and Abbott (*1*) that acid soils may contain much more soluble iron and aluminum than neutral or calcareous soils and may even contain an injurious amount of these compounds.

The following work on the availability of iron compounds is based on the assumption that the chlorosis and the poor growth of rice in the calcareous soils were caused by a lack of available iron. This assumption seems justified by the results presented in the first part of this report.

AVAILABILITY OF ORGANIC IRON COMPOUNDS

In work with pineapples it developed that in the presence of a great amount of organic matter a large amount of carbonate of lime was required to induce chlorosis (*12*). This suggested that in calcareous soils organic iron compounds might be more available than the inorganic, just as iron in solution as a complex ion is less completely precipitated by the usual reagents. The idea seemed substantiated by tests with rice in nutrient solutions containing carbonate of lime, where ferric tartrate furnished much more available iron than equivalent quantities of ferrous sulphate or ferric chlorid.

EXPERIMENT VIII.—Tests were accordingly conducted to determine the effect of various iron compounds and organic materials on the growth of rice in both calcareous and noncalcareous soils. In this experiment the effects of certain pure organic compounds of iron were compared with those of ferric chlorid and ferrous sulphate. A substance which

may be called "ferric molasses" was also used. This was prepared by boiling together 2 parts of ferrous sulphate and 10 parts of a final molasses. It doubtless contained some ferric acetate, glucolate, laevulate, possibly other organic iron compounds, and considerable inorganic iron. As a control on the action of the "ferric molasses," the same quantity of molasses which had been similarly boiled without addition of iron was applied to two other lots of pots. To one of these lots ferrous sulphate was applied after the boiled molasses had been mixed with the soil in the pots designated as "molasses and ferrous sulphate" in Table XI.

Five rice plants were grown in each pot from September 28 to December 28, 1914. In the noncalcareous series each pot contained 14 pounds of loamy soil with the moisture content maintained at 23 per cent of the dry weight; and in the calcareous series each pot contained 14 pounds of loamy soil with the moisture content maintained at 27 per cent of the dry weight. The calcareous soil contained 17.8 per cent of carbonate of lime. A fertilizer consisting of 1.8 gm. ammonium nitrate, 4.2 gm. sodium nitrate, 3 gm. ammonium sulphate, 0.4 gm. acid potassium phosphate, 3.9 gm. acid phosphate, and 3.8 gm. potassium sulphate was added to each pot in four applications. The molasses and all the iron compounds were mixed with the soil before the rice was planted. The iron was applied at the rate of 0.25 gm. and the molasses at the rate of 6.25 gm. per pot. The results of the experiment are summarized in Table XI.

TABLE XI.—*Comparative availability to rice plants of organic and inorganic compounds of iron in a calcareous and noncalcareous soil: Experiment VIII*

Special additions to the soil.	Oven-dried yield of plants per pot.											
	Calcareous soil.						Noncalcareous soil.					
	Series A.	Series B.	Series C.	Series D.	Series E.	Average.	Series A.	Series B.	Series C.	Series D.	Series E.	Average.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm*
	20	16	9	12	13	14	39	41	38	41	59	44
Ferric chlorid	16	18	7	8	15	13	.....	.....	.....	.....	.....	.....
Ferric tartrate	12	21	26	12	26	19	49	55	57	43	52	51
Ferric citrate	18	12	13	23	7	15	51	42	43	42	46	45
Ferric valerianate	11	28	8	7	10	11	49	58	51	44	48	50
Ferric benzoate	9	20	34	18	18	20	49	58	46	52	59	53
Molasses	7	5	2	2	3	4	52	54	41	50	54	50
"Ferric molasses"	18	7	22	15	14	15	42	44	55	50	60	50
Molasses and ferrous sulphate	6	9	4	5	4	6	40	49	54	49	56	50
Ferrous sulphate	16	13	21	15	7	14	.....	.....	.....	.....	.....	.....

Three weeks after planting, all plants in the noncalcareous soil were green, while many plants in the calcareous soil were slightly chlorotic. Those plants in the calcareous soil which received molasses alone or molasses with ferrous sulphate were markedly chlorotic (Pl. 6, B). During later growth the plants in noncalcareous soil remained green and those in calcareous soil became more chlorotic, some plants eventually dying from the top down.

In the noncalcareous soil none of the special compounds affected growth significantly, and in the calcareous soil none of the iron compounds proved efficient sources of iron, although possibly the ferric tartrate and benzoate increased growth slightly.

Molasses alone and molasses followed by ferrous sulphate depressed growth markedly and intensified the chlorosis of plants in the calcareous soil, but the "ferric molasses" had no effect. Probably the molasses that had not been treated with iron still further depressed the availability of iron in the calcareous soils by promoting the formation of insoluble organic iron compounds.

EXPERIMENT IX.—Later a second test was conducted with pure organic iron compounds and organic materials containing iron in calcareous and noncalcareous soils. The pure iron compounds were applied so as to furnish 0.75 gm. or 1.50 gm. of iron per pot, the smaller application being at approximately the same rate as in the preceding experiment, if the sizes of the pots and quantities of soil used in the two experiments are considered. In the tests with ferric citrate and ferric tartrate, a comparison was made between the results obtained by mixing all the material with the soil before planting and those obtained by applying the material in small doses in solution during the growth of the plants. This was done to see if the materials might not be available for a short time in the soil although rendered unavailable in the course of time by bacterial or other action.

The "ferric humate," which, it was thought, might contain some iron compounds similar to those existing in a natural soil, was prepared by extracting leaf mold with 4 per cent ammonia, acidifying with hydrochloric acid, washing the precipitate free from chlorids, and evaporating the precipitate to dryness with sufficient ferric chlorid solution to furnish 25 per cent as much iron as dry matter. The "mixture" used per pot was composed of 4 gm. dried blood, 40 gm. Stizolobium vines, 40 gm. tobacco stems, and 0.90 gm. iron from equal parts of ferric citrate, tartrate, "humate," tannate, oxalate, and benzoate. Velvet beans (Stizolobium) were tested because they are extensively grown as a green manure crop. Both Stizolobium vines and tobacco stems were cut up before mixing with the soil. Citric and tartaric acids were tried to see whether an organic radical alone would have any effect in maintaining available iron in the soil. The test was conducted from December 8, 1916, to February 19, 1917, with eight rice plants in each pot. The pots contained 42 pounds of sandy loam soil, or 47 pounds of sandy soil containing 10 per cent carbonate of lime. The moisture contents of both soils were maintained at 18 per cent of the dry weight. The fertilizer for each pot was given in two applications and consisted of 15 gm. ammonium sulphate, 19.5 gm. acid phosphate, and 6 gm. potassium sulphate. The special additions were mixed with the top 4 inches of soil before the rice was planted, except the solutions of ferric citrate

and ferric tartrate which were applied to the soil every other day. Results of the test are given in Table XII.

TABLE XII.—*Comparative availability to rice plants of organic and inorganic iron compounds in calcareous and noncalcareous soils: Experiment I*

Special additions to the soil.	Amount added.	Oven-dried yield of plants per pot.											
		Calcareous soil.						Noncalcareous soil.					
		Series A.	Series B.	Series C.	Series D.	Series E.	Average.	Series A.	Series B.	Series C.	Series D.	Series E.	Average.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
None		28	22	23	26	26	.....	60	67	68	65	75	.....
None		30	24	17	24	28	25	69	72	70	69	76	69
Ferric oxalate	2.43	18	21	20	24	24	21	78	70	66	54	75	69
Do	4.86	19	20	25	19	24	21	62	66	69	68	65	66
Ferric tannate	8.18	19	22	22	29	25	23	72	68	68	73	69	70
Do	16.36	23	28	23	22	28	25	69	68	77	68	75	71
"Ferric humate"	3.75	18	16	18	18	16	17	61	69	71	63	65	66
Do	7.50	20	16	13	16	16	16	59	61	66	63	56	61
Ferric citrate	8	24	21	24	22	24	23	63	64	95	71	64	65
Solution of ferric citrate		24	20	25	28	26	25	75	75	79	77	69	75
Ferric tartrate	9.34	27	20	22	24	21	23	71	69	74	66	62	68
Solution of ferric tartrate		23	26	15	25	26	23	67	78	72	75	57	70
Tobacco stems	40	28	30	26	33	31	30	62	64	66	72	67	66
Do	80	36	32	37	37	32	34	57	56	58	64	63	60
Stizolobium vines	40	24	26	24	32	25	26	74	60	68	66	70	68
Do	80	38	34	37	32	34	35	67	65	66	67	68	67
Dried blood	4	21	22	19	21	24	21	65	65	69	78	76	71
"Mixture"	92	28	27	26	30	26	27	59	54	57	59	56	57
Citric acid	10	29	23	32	32	23	28	72	68	66	58	77	68
Tartaric acid	10	28	33	26	36	28	30	71	67	72	68	73	70

After three weeks the plants in noncalcareous soil were about twice the size of those in calcareous soil. Later the plants in calcareous soil were all more or less chlorotic, but the plants in pots receiving the larger applications of tobacco stems, cover crop, or "mixture" were less chlorotic than others. All the plants in the noncalcareous soil were a good green throughout growth.

In the noncalcareous soil none of the materials significantly affected growth except the "mixture," which depressed the yield about 20 per cent. In the calcareous soil the "ferric humate" was distinctly injurious, while the larger applications of tobacco stems and Stizolobium vines were plainly beneficial, although they did not induce a normal growth.

SUMMARY.—All organic iron compounds tried in the two preceding experiments failed to increase appreciably the growth of rice in the calcareous soils. It is, therefore, probable that organic iron is no more available than inorganic iron in such soils.

While concentrated or soluble organic materials, such as dried blood, citric and tartaric acids, molasses, and a humus extract, failed to ameliorate the chlorosis, bulky organic materials, such as tobacco stems and velvet bean plants, when used in considerable quantities measurably improved the growth and color of the plants. Also, in previous work with pineapples and sugar cane large amounts of stable manure ameliorated

or completely overcame the chlorosis, although small amounts were without appreciable effect.

In view of the nonavailability of the concentrated organic iron compounds, it seems probable that the beneficial effect of the bulky organic materials was not due primarily to the addition of certain iron compounds that were available in the calcareous soil as a whole. It is more probable that the particles of organic material formed isolated centers or points where iron was more available than in the rest of the soil. The plants were not able to secure all the iron they needed from these points for the reason that plants are apparently not able to absorb a maximum amount of iron with only a portion of their roots (*18*).

It may seem that the results of the last two tests negative the conclusions arrived at in the experiments with rice grown in solutions containing carbonate of lime where organic iron compounds supplied sufficient available iron. Conditions in the nutrient solutions, however, were somewhat different from those in the soil. To begin with, in the nutrient solutions the plants obtained their iron from an ordinary solution that was more or less sterile and that was frequently renewed. In the soil, on the other hand, the plants probably obtained their nutrients from aqueous films surrounding the soil particles, and there is evidence that in films reactions may occur which do not take place in ordinary solutions. Furthermore, bacterial action in the soil might have destroyed rapidly certain of the organic compounds supplied.[1]

EFFECT OF WATER CONTENT OF SOIL ON THE AVAILABILITY OF IRON

At present we know little of the true soil solution or film moisture. It is evident, however, that the nature of the soil particles must influence the composition of the solution or substances dissolved in the enveloping film. In the films surrounding particles of calcium carbonate the amount of iron in solution must be greatly reduced, since the iron would be precipitated as ferric oxid.

If it is assumed that each particle in the soil is isolated and that the moisture films surrounding the individual particles are discontinuous, it would follow that the larger the proportion of particles which were carbonate of lime the less soluble iron there would be in the whole medium.

This assumption would explain why carbonate of lime is more effective in inducing chlorosis the more finely divided it is and why a certain quantity of carbonate of lime exerts a stronger influence in a sandy soil containing relatively few particles than in a clay soil containing a large number of particles.

However, the case is not so simple as is assumed above. The moisture films are not discontinuous but more or less continuous, the continuity

[1] The fact that ferric citrate and ferric tartrate were no more effective when applied in frequent small doses than when applied all at once is some evidence against the idea that the organic iron compounds were unavailable because they were destroyed by bacterial action.

and thickness of the films depending somewhat on the amount of moisture in the soil. The substances in solution in a film surrounding one particle will therefore react with those in films surrounding adjacent films. One particle of carbonate of lime would affect the soluble iron in the films of a certain number of adjacent particles.

While the moisture films are to a certain extent continuous, we know that the composition of the films is not uniform throughout the soil. This is evident from certain well-established facts, such as the slight lateral movement of fertilizers. If the composition of the films were uniform and conditions were analogous to those in a solution with relatively few solid particles, a slight amount of carbonate of lime would have the same effect as a much larger amount. This, however, is not the case.

It might be expected that the effect of carbonate of lime in depressing the availability of iron and in inducing chlorosis would be influenced somewhat by the amount of water in the soil, since the aggregation of the soil particles and their moisture films would be affected by the water content. It was, therefore, of interest to observe the manner in which the growth and chlorosis of rice would be affected by different percentages of moisture in calcareous soil.

A preliminary test was conducted with four pots, each of which held 36 pounds of soil containing 15 per cent of calcium carbonate. Twelve rice plants were grown in each pot with abundant fertilizer. The plants were grown 30 days with 22 per cent of moisture in the soil. Water was then added to two of the pots until there were 2 inches of water above the surface of the soil, and the other two pots were maintained unchanged at 22 per cent moisture. After 67 days' growth the plants were cut.

The plants in all four pots were very slightly chlorotic at 30 days, but a few days after the extra water was added the submerged plants became intensely chlorotic and remained so for about 10 days. They then quickly improved in color, and a few days later the submerged plants were a perfectly normal green, while the plants in the soil with 22 per cent moisture were markedly chlorotic. This difference persisted until the plants were cut. The plants grown for the whole period with 22 per cent moisture gave an average green weight of 175 gm. per pot, while the plants grown for 30 days with 22 per cent moisture and then submerged for 37 days yielded 424 gm. per pot.

EXPERIMENT X.—An extended test was conducted from January 2 to March 22, 1918, using one noncalcareous soil and two calcareous soils (one a beach sand with practically no organic matter and the other a loam).[1] The noncalcareous soil was used as a control to determine how the growth of rice would be affected by different amounts of water in a

[1] The calcareous loam was the same as the noncalcareous soil except for the addition of the carbonate of lime some years before.

soil adapted to its growth. Each pot received 9 gm. sulphate of potash, 6 gm. double superphosphate, and 22.5 gm. sulphate of ammonia divided in two applications. Twenty rice plants were planted in each pot, but these were thinned to 10 when growth was well established. The results are given in Table XIII.

TABLE XIII.—*Effect of varying degrees of moisture on the availability of iron to rice plants in calcareous and noncalcareous soils*

Soil No.	Kind of soil.	Percentage of calcium carbonate.	Optimum water content of soil expressed as percentage of dry weight of soil.	Maximum water capacity of soil expressed as percentage of dry weight of soil.	Amount of soil per pot.	Amount of water maintained in soil during growth of plant.	Oven-dried yield of plants per pot.			
							Series A.	Series B.	Series C.	Average.
					Pounds.		*Gm.*	*Gm.*	*Gm.*	*Gm.*
						22.3 per cent	119.8	122.1	103.9	115.3
						26.3 per cent	125.4	120.8	127.0	124.4
						30.3 per cent	146.1	128.9	137.7	137.6
1647	Loam	.....	25.5	34.3	69	34.3 per cent	129.2	142.1	141.6	137.6
						Water at surface of soil	159.9	167.5	153.8	160.4
						Water 3 inches above surface of soil	155.9	157.0	177.6	163.8
						20.2 per cent	58.1	51.5	57.5	55.7
						24.2 per cent	74.9	68.9	79.1	74.3
						28.2 per cent	53.7	70.8	78.8	67.8
1648	...do...	8.53	23.2	36.2	69	32.2 per cent	66.1	74.2	67.1	69.1
						36.2 per cent	87.3	72.9	77.8	79.3
						Water 3 inches above surface of soil	112.5	134.6	122.8	123.3
						11 per cent	12.4	9.9	9.2	10.5
						18 per cent	6.1	13.4	13.6	11.1
1194	Sand	19.0	11.6	25.0	98	25 per cent	1.2	9.4	1.4	4.0
						Water 3 inches above surface of soil	17.6	8.4	28.1	18.1

The different water contents maintained during the experiment were made up when the plants were 4 days old, except that the pots to receive 3 inches excess water were made up with water at the surface at this time, the water being raised to 3 inches when growth permitted it. When 11 days old, the plants in soils No. 1647 and 1648, where water was at the surface or above it, were markedly chlorotic, as well as all the plants in soil No. 1194. After 31 days' growth, all the plants in soil No. 1194 were still markedly chlorotic; the submerged plants in soil No. 1647 were normal green and were growing rapidly, as were all other plants in this soil; in soil No. 1648 the submerged plants and those in pots with 20.2 and 24.2 per cent water were normal green, while those in pots with 28.2, 32.2, and 36.2 per cent water were plainly chlorotic. At 72 days' growth, when the plants were cut, the appearance in regard to chlorosis was similar to that at 31 days, except that in soil No. 1194 the few plants that had not died in the pots with 3 inches excess water were normal green and far larger than the others.

The temporary chlorosis affecting the plants where the excess water was added is entirely distinct from the lime-induced chlorosis. A similar yellowing takes place in the field when the fields are flooded following

early growth without submergence. Several of the surplus plants in the pots with excess water were brushed repeatedly with ferrous sulphate, but the treatment did not improve the color of the plants in the slightest. Evidently this particular chlorosis is not due to lack of iron. Doubtless when the water content of the soil is raised above the point of saturation the old roots are unable to function properly and the nutrition of the plant is disturbed until new roots are sent forth which are able to function under the new conditions.

It was thought that roots of the submerged plants might show morphological differences from roots of plants grown with ordinary amounts of water in the soil. Samples of roots from plants grown in soil No. 1647 were therefore subjected to a preliminary examination by Dr. Albert Mann, of the Bureau of Plant Industry, United States Department of Agriculture, to whom thanks are due. A portion of Dr. Mann's report of the preliminary examination follows:

> The differences noted between No. 1805 with 24.2 per cent moisture, 1807 with 32.2 per cent, and 1809 with water standing three inches above the surface are slight. There is in general more compactness and strength of tissue in 1805 than in the others. The central fibrovascular bundle mass is larger in proportion to the cortex than in 1807 or 1809. The cells of all the tissues are slightly more robust. The light parenchyma, which makes up the cortex from the endodermal ring to the epiderm, is especially thinner walled and more developed in 1809. There is also a notable absence of root hairs in this sample as compared with the other two, which is, of course, the inevitable result of the roots growing submerged in water.

The series in the noncalcareous soil shows that the growth of rice should increase regularly with increasing amounts of water in the soil until a percentage near the saturation point of the soil is reached and that, possibly because of a different root growth, there should be another considerable increase when enough water is added for submergence. In No. 1648, however, the series with the calcareous soil, there were two maxima of growth, one at 24.2 per cent water and one at 3 inches excess; and in the calcareous sand No. 1194 there were also two maxima. It is believed that the first lower maximum was due to iron being a little more available at that water content than at a higher content. The great increase in growth in the calcareous soils produced by submergence [1] was probably due chiefly to the fact that the modified roots are better able to assimilate iron than the ordinary type of root and was probably not due to increased availability of iron in the submerged soil.

It is felt that the results substantiate the idea that the availability of iron in the soil is affected somewhat by the amount of water in the soil, the availability being slightly greater near the optimum water content than with larger amounts.

The effect of the water content is probably due to its influence on the extent to which reactions take place between the moisture films

[1] It will be noted that in the calcareous soils the increase produced by submergence was much greater than in the noncalcareous soil.

surrounding the calcareous particles and those surrounding the other soil particles. With moisture contents above the optimum the moisture films become more continuous and the sphere of influence of the particles of carbonate of lime in reducing the availability of iron becomes more extended.

Incidentally the tests established a fact of considerable practical importance—namely, that rice may be expected to make a practically normal growth in certain calcareous soils if the soils are submerged.

SUMMARY

There are a few plants which are generally conceded to be calcifugous, inasmuch as they are rarely found on calcareous soils.

Soil surveys of several species of cultivated plants show that a particular type of chlorosis affecting these plants occurs only on calcareous soils. All calcareous soils, however, do not induce chlorosis in these plants.

Addition of carbonate of lime to soils producing normal, calcifugous plants causes the soils to produce chlorotic plants.

It is, therefore, evident that a chlorosis of some plants is caused by, or is associated with, the presence of carbonate of lime in the soil.

The weight of the evidence from ash analyses of chlorotic plants seems to point to a deficiency of iron in the ash as being one cause of the chlorosis, with possibly an excess of lime as a contributory cause.

Treatment of chlorotic plants with iron shows that a lack of iron in the plant is at least one of the causes of lime-induced chlorosis.

There is no evidence of a general "lime effect" in inducing chlorosis, the different lime compounds affecting the plants differently.

Rice, one of the plants sensitive to lime, does not appear to be sensitive to the alkalinity of carbonate of lime except as this alkalinity influences the availability of the iron.

Lime-induced chlorosis seems to be due simply to a depression in the availability of iron in calcareous soils.

A number of pure organic iron compounds and concentrated organic preparations proved to be inefficient sources of iron for rice in calcareous soils. Bulky organic compounds such as stable manure, velvet bean plants, and tobacco stems, when used in considerable quantity, however, enabled the plant to secure more iron.

The availability of iron in calcareous soils appears to be slightly greater near the optimum water content of the soil than at higher percentages of water.

Although rice becomes chlorotic in calcareous soils with ordinary percentages of water, it will grow normally in certain calcareous soils if the soil is submerged. This is believed to be due to the growth, under submerged conditions, of a new kind of root that is better able to assimilate iron than the root formed in the soil with less water.

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PLATE 5

A.—Rice grown in calcareous and noncalcareous soils and sprayed with ferrous sulphate solution (experiment I).

1–4. Noncalcareous soil; plants in 1 and 3 unsprayed, those in 2 and 4 sprayed.

5–8. Soil containing 30 per cent carbonate of lime; plants in 5 and 7 unsprayed, those in 6 and 8 sprayed.

B.—Apparatus used in growing plants in experiment VII.


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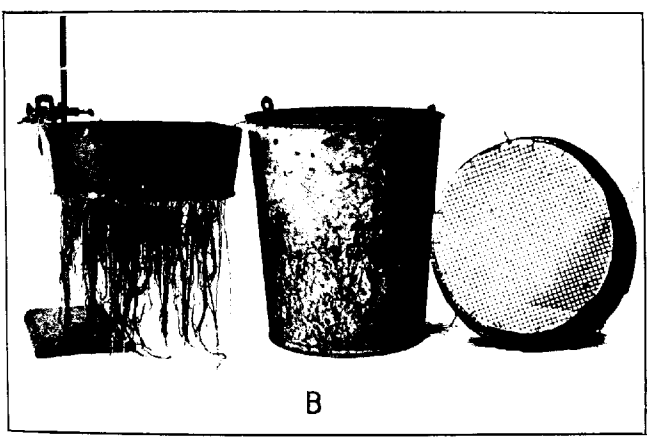
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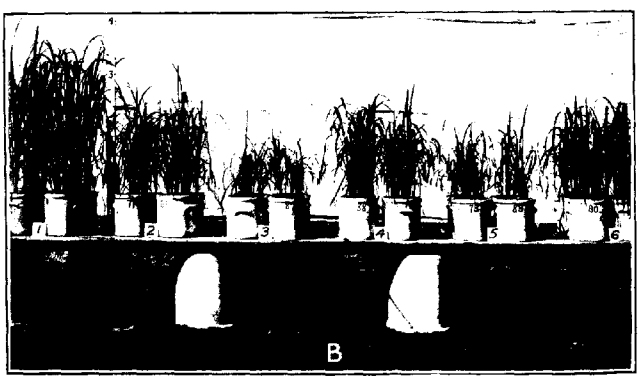
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PLATE 6

A.—Effect of carbonate of lime in depressing the availability of iron (experiment VII).

1. Calcareous soil in bucket, silica sand plus iron in sieve.
2. Calcareous soil in bucket, silica sand plus carbonate of lime and iron in sieve.

B.—Effect of various substances on growth of rice in calcareous soil (experiment VIII).

1. Noncalcareous soil.
2. Calcareous soil.
3. Calcareous soil with molasses added.
4. Calcareous soil with "ferric molasses" added.
5. Calcareous soil with molasses and ferrous sulphate added.
6. Calcareous soil with ferrous sulphate added.

AN EXPERIMENTAL STUDY OF ECHINACEA THERAPY

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INTRODUCTION

The use of echinacea as a remedy for various disorders in both human beings and animals is said to have originated with the American Indians, from whom the early settlers in the West learned of the "virtues" of the plant.[1]

In 1885 Dr. H. C. F. Meyer, of Pawnee City, Nebr., sent a specimen of the plant to Prof. Lloyd. It was identified as *Echinacea angustifolia* (DC.). Dr. Meyer was using the root of this plant in a secret mixture which he called "Meyer's Blood Purifier," and the claims which he made for the curative properties of the root are described as "most exaggerated." Indeed, he had such confidence in it that he offered to submit to repeated bites of rattlesnakes, proposing to demonstrate the remedial power of echinacea against this venin by using his preparation of the root as an antidote. This offer was, of course, refused, but the drug was studied by Dr. John King, Prof. H. T. Webster, and others, with the result that clinical evidence was obtained which appeared to substantiate some of the claims of Dr. Meyer. Preparations of the plant were placed on the market, and the medicinal use of echinacea increased rapidly. Many physicians have reported favorable results from its use in various diseases.

In 1909 a report (*3*) of the Council on Chemistry and Pharmacy of the American Medical Association denied echinacea a place in "New and Non-Official Remedies" and directed suspicion at the value of the drug, stating:

> In view of the lack of any scientific scrutiny of the claims made for it, echinacea is deemed unworthy of further consideration until more reliable evidence is presented in its favor.

In spite of this the use of echinacea has become extensive. Lloyd states that it is used in larger quantities than any other American drug introduced since 1887. The fluid extract and tincture are made in enormous quantities, and the root enters into the composition of a large number of patent, proprietary, and nonsecret mixtures.

The last revision of the National Formulary includes a description of echinacea and furnishes a formula for its fluid extract (*1, p. 74, 294*). This amounts to a quasi official recognition of the drug. It has never been official in the United States Pharmacopœia.

[1] The historical facts about echinacea have been obtained almost wholly from published accounts by Meyer (*15*) and Lloyd (*11, 12, 13*). Reference is made by number (italic) to "Literature cited," p. 83–84.

Five species of echinacea are mentioned in works on botany (*6*). *Brauneria purpurea* (DC.) (*Rudbeckia purpurea* or *Echinacea purpurea* [Moench]) is found from western Pennsylvania and Virginia to Michigan, Iowa, and southward. *B. angustifolia* (DC.) (*E. angustifolia* [DC.]) is found from Tennessee and Minnesota to Saskatchewan, Texas, and Nebraska. *B. pallida* (Nutt.) occurs from Michigan and Illinois to Texas and Alabama, while *B. paradoxa* (Norton) and *B. atrorubens* (Britton) (*R. pallida*) are found from Missouri to Texas. The rays of the last two species are bright yellow in color. The species which furnish the larger proportion of the medicinal supplies are *B. pallida* and *B. angustifolia*. It has been stated that the eastern species, *B. purpurea*, is inert.

CHEMICAL CONSTITUENTS

In 1897 Lloyd (*11*) reported the presence of a colorless alkaloid and a colorless acid-reacting substance of intensely acrid properties. The root has been subjected to analysis by Heyl and Staley (*8*) and Heyl and Hart (*7*), by whom the alkaloid was identified as betaine. Nothing of a physiologically active nature, however, was isolated by these investigators.

THERAPEUTIC USES

General accounts of the various uses to which echinacea has been put have been published by Ellingwood (*4*) and by Lloyd (*13*). Echinacea is stated to be a corrective of "depravation" of body fluids, of septic, fermentative, or zymotic conditions. It is said to antagonize infectious processes and "blood poison," to be useful in puerperal sepsis, uremia, pernicious malarial or septic fevers, typhoid fever, and all fevers caused by absorption of septic material. It has been recommended as a specific against the venins of rattlesnakes, other serpents, and insects (*9*) —

in crotalus it stands without a peer.

Pyemia, goiter, smallpox, anthrax, and hydrophobia are reported to have been cured by echinacea. It is said to be an antidote for tetanus. It has been used locally in erysipelas, bedsores, fever sores, chronic ulcers, glandular indurations, syphilitic nodules, burns, and gangrene (*14*) and is said to be an active sialogogue, diuretic, and diaphoretic. Jensen found it useful in the treatment of carbuncles.

The uses of echinacea in veterinary practice have been discussed by Fish (*5*), who found the root to increase the elimination of urea. In some pharmacological experiments upon kittens he obtained evidence of narcosis, and emesis was provoked by the fluid extract given per os. He quotes five cases in which the administration of echinacea was followed by improvement.

The compound of inula and echinacea prepared especially for parenteral administration has been stated to be useful in the treatment of tuberculosis (*18*), has been designated "an effective treatment for canine

distemper," and is recommended in the treatment of equine influenza (*10*). Slawson (*16*) does not consider this preparation satisfactory in the treatment of canine distemper. He finds that its action does not differ from that of nuclein, leucocyte extract, or plain serum.

PRESENT INVESTIGATION

The investigation of which the results are here reported was undertaken for the purpose of determining, so far as the limits of laboratory experiment permit, the usefulness of echinacea as a remedy in several pathological conditions induced by bacteria, their products, or allied toxins.

The animals used were guinea pigs bred at the Bethesda (Md.) Experiment Station of the Bureau of Animal Industry, all in healthy condition and apparently normal. The animals were kept under observation long enough before experimental use to exclude any but the most remote possibilities of accidental factors.

PREPARATIONS TESTED

The preparations of echinacea employed in the remedial work consisted of the following:

1. A sample of "Specific Medicine Echinacea," manufactured by and obtained from Lloyd Brothers, of Cincinnati, Ohio. This is a liquid preparation which is stated to contain 480 gr. of echinacea root per fluid ounce, or slightly more than a modern fluid extract. It contained 69 per cent of alcohol and conformed to the organoleptic tests for select echinacea. It was identified and preserved free from change during the whole course of the investigation. This remedy was diluted with distilled water for administration per os. The treatment caused the mixture to become cloudy because of the suspension of the resinous and oily constituents of the plant. These mixtures were never allowed to stand long enough for the insoluble matters to separate but were given to the animals while still in the stage of emulsion. In this way it is certain that the guinea pigs received all of the constituents of echinacea which are soluble in 69 per cent alcohol.

2. A fluid extract of echinacea purchased on the open market. This contained 70 per cent of alcohol and was identified, preserved, and administered exactly as was the specific medicine mentioned above.

3. "Subculoyd Inula and Echinacea," manufactured by and obtained from Lloyd Brothers. This liquid was used in the greater portion of the parenteral administrations. It is stated to contain, in 3 mils, 1.33 mils of *Inula helenium* and 1 mil of echinacea. It does not contain alcohol. This material was scrupulously preserved from contamination and change. In certain of the experiments it was administered intra muscularly; in other cases it was injected subcutaneously. Upon

autopsy of animals treated with this liquid there was noticed some necrosis of the tissues at the points of injection, but no other unfavorable results from its administration were observed.

Certain other preparations of echinacea which are sometimes used were not tested. A tincture of the green root is on the market, as is also a variety of powdered and solid extracts of echinacea. These preparations are all made with a menstruum of strong alcohol, and it is therefore not to be supposed that they contain any components not present in the fluid extracts which we used. The manufacturers of certain green-root tinctures assert that this product is superior to preparations of the dried root; there is, however, not the slightest published evidence to substantitate this assertion. The early settlers are said to have used the green root bruised and in the form of infusion. In the present work no such form of the remedy was used. It is quite possible that an infusion would contain some substances which are absent in the strongly alcoholic preparations and might, on this account, affect the organism differently. The claims of the therapeutic efficiency of echinacea have, however, been very largely made through the use of alcoholic preparations, and we therefore felt justified in employing these in determining its value as a remedy.

PATHOLOGICAL CONDITIONS TREATED

The acute experimental pathological conditions produced in the guinea pigs were tetanus, botulism (in both of which the diseases were produced by bacterial toxins), anthrax, septicemia (in both of which the bacteria were injected into the animals), and crotalus poisoning (in which the venin of rattlesnakes was injected). The chronic conditions were those of tuberculosis, which was produced by inoculation with the bacillus, and a trypanosomiasis (dourine), produced by inoculation with the trypanosomes. The sources of these materials and the methods of injection are described in the part of this paper which reports the experimental work.

METHODS

The methods employed for testing the remedial powers of echinacea against these several conditions were as follows:

1. Animals were injected with the pathogenic material and were immediately afterwards treated with echinacea, in suitable doses, one dose per diem, until the animal succumbed or became unable to swallow (if the administration was per os).

2. Animals were dosed with echinacea for several days before they were injected with pathogenic material, a protective treatment designed to favor the drug as much as possible, and were given remedial doses as long after the injection as possible. The treatment with the "Subculoyd" followed the same course. Treatment was necessarily suspended on Sundays and holidays, but in all except the chronic cases the time was so chosen as to minimize breaks due to such cause.

DOSAGE

The dose of fluid extract echinacea is variously given as from 10 minims to 0.5 fluid ounce for adult human beings, and for the "Subculoyd" preparation the parenteral dose recommended is 3 to 10 mils daily. It has also been stated that large doses of echinacea do not produce toxic effects upon healthy subjects, although this has been contradicted. The doses chosen for our experimental animals ranged from 0.25 to 1 mil daily of fluid extract and from 0.2 to 0.5 mil daily of subculoyd, which, calculated on a kilogram-of-body-weight basis, would correspond to from 40 to 160 mils daily of fluid extract and from 30 to 60 mils daily of the "Subculoyd" for man. It is well known, however, that to produce a given effect in guinea pigs requires very much larger doses per kilo than in larger animals. We decided upon a large dose of the remedy so as to favor the echinacea as much as possible and to remove any possibility of failure through administration of inadequate amounts.

GENERAL RESULTS AND CONCLUSIONS

In no one of the diseases treated with echinacea was any evidence obtained to show that the plant exerts any influence upon the course of infectious processes under laboratory conditions. Daily feeding of animals with echinacea preparations for several days before injection of microorganisms or their toxins did not increase the resistance of the animals to these agents. In the two chronic cases where the animals were given doses of echinacea preparations for extended periods of time nothing appeared in the autopsy pictures which could be attributed to the action of the echinacea per se, except that in two cases a gastric catarrh was present which may have been due to this plant. In all cases the course of the disease was the same in the control animals and in the animals which were given remedial treatment.

It does not appear, therefore, that echinacea or the preparation of inula and echinacea are of value in the treatment of diseases produced by microorganisms and their toxic products.

EXPERIMENTAL WORK

I.—TESTS OF ECHINACEA AS A REMEDY FOR TETANUS

In order to test the efficacy of echinacea as a remedy for tetanus a total of 29 guinea pigs was used. The animals were injected with a sample of standard tetanus toxin furnished by the Hygienic Laboratory of the United States Public Health Service. This material was kindly placed at our disposal by Dr. W. N. Berg, of our laboratory, who had used a part of it in his work on the destruction of tetanus antitoxin by chemical agents (*2*). It had been carefully standardized; the minimal

lethal dose was 0.0007 mgm. for a 350-gm. guinea pig. The material was preserved in vacuo in the dark and at low temperature. A fresh solution of the toxin was prepared for use by carefully weighing out a small quantity and dissolving this in just enough sterile normal salt solution to furnish a liquid which should contain 6 minimal lethal doses per mil. Each of the experimental animals received 0.5 mil of this solution, an equivalent of 3 minimal lethal doses.

EXPERIMENT 1.—ECHINACEA ADMINISTERED PER OS

Four guinea pigs were each given a 3-mil dose of a mixture of 1 mil of the "Specific Medicine Echinacea" and 2 mils of distilled water once a day for six days, a total of 6 mils of the remedy. The animals were rested one day and on the eighth day were given another dose of the remedial mixture, immediately followed by a subcutaneous injection of 0.5 mil of tetanus toxin solution (3 minimal lethal doses). On the following day all the animals received a dose of the remedy, so that each guinea pig had then received a total of 8 mils of specific echinacea, equivalent to somewhat more than 8 gm. of the root.

All of the animals exhibited the typical symptoms of tetanus and died on the ninth day. The autopsies were negative; no evidence of any ntercurrent disease was obtained. Three control guinea pigs which were injected at the same time as the experimental animals died on the same day with symptoms of tetanus and furnished the same post-mortem

EXPERIMENT 2.—ECHINACEA INJECTED INTRAMUSCULARLY

Echinacea injected intramuscularly was tested upon five guinea pigs. The undiluted "Specific Medicine Echinacea" was injected into the right and left thighs on alternate days. Each animal received four 0.5-mil doses, one per day, a total of 2 mils. The treatment caused considerable swelling at the points of injection. On the fourth day the animals were all given subcutaneous injections of 0.5 mil of the tetanus toxin solution. They all exhibited the characteristic symptoms of tetanus and died early in the morning of the third day after the injection. The autopsy showed considerable local reaction of the tissues to the injection of the echinacea. This consisted of a sero-sanguineous infiltration of the subcutaneous and muscular tissues with small areas of degeneration in the musculature at the point of injection. The internal organs showed no gross lesions.

EXPERIMENT 3.—ECHINACEA AND TOXIN ADMINISTERED SIMULTANEOUSLY

In order to determine whether echinacea possesses properties similar to the antitoxins, five guinea pigs were injected subcutaneously with 0.5 mil of the tetanus toxin solution and immediately received 0.5 mil of undiluted "Specific Medicine Echinacea" injected intramuscularly into

the right thighs. On the following day 0.5 mil of the remedy was injected into the left thighs of the animals. This treatment was wholly remedial, no protective doses having been given as in experiments 1 and 2 of this series. In three days after the injection of the toxin all the animals were dead after exhibiting typical tetanus. The autopsy picture was similar to that in experiment 2.

EXPERIMENT 4.—INULA AND ECHINACEA INJECTED INTRAMUSCULARLY

Protective doses of the "Subculoyd Inula and Echinacea" were injected intramuscularly into five guinea pigs. The dose administered was 0.5 mil per day for six days, a total of 3 mils, corresponding to 1 gm. of echinacea and 1.33 gm. of inula. On the eighth day after the treatment was begun the animals were injected with 0.5 mil of tetanus toxin solution, and a dose of 0.5 mil "Subculoyd" was given. The total dose of the remedy was 3.5 mils. On the following day all the guinea pigs showed typical symptoms of tetanus, and one died; the remaining four died the next day. On autopsy there was found a moderately severe local reaction of the tissues to the injection of the inula and echinacea. The subcutaneous and muscular tissues at the site of injection showed considerable hemorrhage and sero-sanguineous infiltration. No gross lesions were apparent in any of the internal organs.

EXPERIMENT 5.—INFLUENCE OF ALCOHOL ON TETANUS

Since the "Specific Medicine Echinacea" employed in the foregoing experiments contained 69 per cent of ethyl alcohol, it was considered desirable to study the influence of this factor upon tetanus under the conditions of the echinacea experiments. Accordingly, a mixture of alcohol and distilled water was made which contained exactly 69 per cent of alcohol, and this was injected intramuscularly into four guinea pigs in 0.5-mil doses. Each guinea pig received two doses, one into the right thigh and, on the next day, one into the left thigh. Two days afterwards all four received 0.5 mil of tetanus toxin solution subcutaneously. In three days two of these animals died, and the remaining two died during the following night. All showed typical symptoms of tetanus. The autopsy showed some congestion of the subcutaneous tissues at the points of injection of the alcohol, hemorrhage in the musculature, and evidence of local degeneration of the muscles. No gross lesions were apparent in any of the internal organs.

EXPERIMENT 6.—CONTROLS

The six control animals were kept under the same conditions as the experimental animals and received the same amounts of tetanus toxin. They all developed the typical symptoms of tetanus and died in less

than three days. The autopsies were negative. No evidence of an intercurrent disease was obtained.

The results of this series of experiments are given in Table I.

TABLE I.—*Results of experiments with echinacea in the treatment of tetanus*

Experiment No.	Guinea pig No.	Weight of guinea pig.	Total dose of remedy.	Dose of toxin.	Effect.	Termination.	Number of days sick
		Gm.	*Mils.*	*M. l. d.*[a]			
1.....	18	395	7	3	Tetanus.....	Died.....	2.
	19	380	7	3	.....do.....	...do.....	2.
	21	335	7	3	.....do.....	...do.....	2.
	22	370	7	3	.....do.....	...do.....	2.
2.....	1	445	2	3	.....do.....	...do.....	Less than 3.
	2	455	2	3	.....do.....	...do.....	Do.
	3	490	2	3	.....do.....	...do.....	Do.
	4	440	2	3	.....do.....	...do.....	Do.
	5	445	2	3	.....do.....	...do.....	Do.
3.....	13	455	1	3	.....do.....	...do.....	3.
	14	470	1	3	.....do.....	...do.....	
	15	435	1	3	.....do.....	...do.....	
	16	450	1	3	.....do.....	...do.....	
	17	460	1	3	.....do.....	...do.....	
4.....	26	490	4	3	.....do.....	...do.....	3.
	27	470	4	3	.....do.....	...do.....	3.
	28	455	4	3	.....do.....	...do.....	3.
	29	400	4	3	.....do.....	...do.....	4.
	30	445	4	3	.....do.....	...do.....	2.
5.....	9	590	1	3	.....do.....	...do.....	4.
	10	480	1	3	.....do.....	...do.....	4.
	11	410	1	3	.....do.....	...do.....	3.
	12	390	1	3	.....do.....	...do.....	3.
6 (controls).	6	415	0	3	.....do.....	...do.....	Less than 3.
	7	480	0	3	.....do.....	...do.....	Do.
	8	440	0	3	.....do.....	...do.....	Do.
	23	475	0	3	.....do.....	...do.....	2.
	24	435	0	3	.....do.....	...do.....	2.
	25	395	0	3	.....do.....	...do.....	2.

[a] Minimal lethal dose.

SUMMARY OF EXPERIMENTS WITH TETANUS

"Specific Medicine Echinacea" was administered to guinea pigs both per os and intramuscularly, the "Subculoyd Inula and Echinacea" was administered to guinea pigs intramuscularly, and 69 per cent alcohol was injected intramuscularly into guinea pigs, as a means of treatment for tetanus. All of these animals were injected with 3 minimal lethal doses of standard tetanus toxin in solution, some animals being injected several days after they had been treated with echinacea, while others were injected first and then treated with echinacea. Neither the protective treatment nor the remedial treatment nor a combination of the two appeared to influence the course of the disease, as all the experimental animals acted in the same way and died in the same time as the controls. From these results it does not appear that echinacea possesses remedial value against experimental tetanus in laboratory animals.

II.—TESTS OF ECHINACEA AS A REMEDY FOR BOTULISM

Since echinacea did not appear to influence the action of tetanus toxin, it was thought desirable to compare its action against another bacterial toxin. For this purpose botulinus toxin was chosen. The material used to produce botulism in the experimental animals consisted of a germ-free filtrate of a glucose beef infusion culture of *Bacillus botulinus* (Boise strain) (*17*) incubated for one month at 37° C. The filtrate was diluted with sterile normal salt solution in such amount that 1 mil was equivalent to very nearly 10 minimal lethal doses for a 400-gm. guinea pig. This toxin was not injected into the animals but was fed through the mouth in order to duplicate the conditions under which this type of poisoning usually occurs.

EXPERIMENT 1.—ECHINACEA ADMINISTERED PER OS

Three guinea pigs only were used, because the results of the experiment were so free from uncertainty that it was not considered necessary to sacrifice more animals in order to determine the facts. The animals were all given 2-mil doses daily of a mixture of 0.5 mil fluid extract echinacea and 1.5 mils distilled water for 6 days. The total protective dosage was 3 mils of the fluid extract, equivalent to 3 gm of echinacea. The animals were rested one day, and on the eighth day after the beginning of the experiment all received 1 mil (10 minimal lethal doses) of botulinus toxin immediately after receiving a 2-mil dose of the remedial mixture. On the following day all the animals were sick. No. 78 received a remedial dose of 2 mils of the echinacea mixture. No. 79 received 1 mil of the same mixture, which was all that it could swallow. With No. 80 the symptoms of pharyngeal paralysis were so marked that it was considered inadvisable to drench the animal on account of the danger of strangulation. This animal died during the afternoon. The remaining two were found dead in the morning of the second day after. The treated pigs and the controls showed no differences. The autopsy showed general hyperemia of the internal organs; there was no evidence of any intercurrent disease.

EXPERIMENT 2.—CONTROLS

Two guinea pigs were used as controls. These animals were fed a 1-mil dose of botulinus toxin (10 minimal lethal doses) on the same date as the experimental pigs. In about 18 hours both animals showed symptoms of botulism; one died in 23 hours after the dose; the other was found dead in the morning of the third day after the dose. The post-mortem findings were similar to those for the experimental animals. The results are summarized in Table II.

TABLE II.—*Results of experiments with echinacea in the treatment of botulism*

Experiment No.	Guinea pig No.	Weight of guinea pig.	Total dose of remedy.	Dose of toxin.	Effect.	Termination.	Number of days sick.
		Gm.	*Mils.*	*M. l. d.*			
1	78	345	4	10	Sick	Died	3
	79	370	3.75	10	do	do	3
	80	405	3.5	10	do	do	1
2 (controls)	81	390	0	10	do	do	3
	82	365	0	10	do	do	1

SUMMARY OF EXPERIMENTS WITH BOTULISM

Fluid extract echinacea was administered per os to guinea pigs for a total of six protective doses. The animals were then fed botulinus toxin. The treatment with echinacea was continued as long as the animals were able to swallow. All the experimental animals developed positive symptoms of botulism and died within three days after ingesting the toxin. From this it does not appear that echinacea possesses remedial value against botulism.

III.—TESTS OF ECHINACEA AS A REMEDY FOR SEPTICEMIA

Twelve guinea pigs were used in testing the remedial value of echinacea in septicemia. The pathogenic material was a 48-hour-old glycerin-agar culture of *Bacillus bovisepticus* of only moderate virulence for laboratory animals. A faintly cloudy suspension of the organisms in sterile normal salt solution was prepared and used for inoculation. While no attempt was made to determine the minimal lethal dose of this organism for guinea pigs, a few preliminary tests undertaken indicated that the dose employed in the following experiments was not excessive.

EXPERIMENT 1.—ECHINACEA ADMINISTERED PER OS (PROTECTIVE)

In order to determine whether the administration of echinacea would increase the resistance of the organism to septicemia if given sufficient time to develop immunity, two guinea pigs were given four daily doses of 3 mils of a mixture of 1 mil of "Specific Medicine Echinacea" and 2 mils of distilled water. The total protective dose was 4 mils, all administered per os. The animals were then allowed to rest for 11 days, when they were injected subcutaneously with 0.5 mil *Bacillus bovisepticus* suspension. Both animals became sick; one died in three days and the other in five days. Two of the controls died in three days and the third control survived. The autopsy showed septicemia.

EXPERIMENT 2.—ECHINACEA ADMINISTERED PER OS (REMEDIAL)

Two guinea pigs were given two daily doses per os of 3 mils of the diluted echinacea mixture used in experiment 1. On the third day they were injected with 0.5 mil of *Bacillus bovisepticus* culture, and immediately afterwards were given a 3-mil dose of the echinacea mixture per os. On the following day both animals were very sick. They were given a fourth dose of 3 mils of the echinacea mixture per os. The total dose was 4 mils of specific echinacea, equal to 4 gm. of the root. Case 65 died in 24 hours and case 66 in 48 hours. The autopsy showed septicemia; typical organisms were demonstrated in blood and organs.

EXPERIMENT 3.—INULA AND ECHINACEA INJECTED INTRAMUSCULARLY (PROTECTIVE)

This experiment was conducted exactly like experiment 1 of this series except that the "Subculoyd" preparation was used instead of the "Specific Medicine Echinacea." Three guinea pigs were given four daily doses of the "Subculoyd" preparation, 0.5 mil being injected intramuscularly, first into the right and then into the left thigh. The total dose was 2 mils. The animals were allowed to rest 11 days and then were injected subcutaneously with 0.5 mil of *Bacillus bovisepticus* culture. All became sick. Case 62 died in 6 days after the inoculation, case 64 died in 12 days, and case 63 survived, being discharged as recovered 10 weeks after the injection. The autopsies on the fatal cases revealed typical pictures of septicemia, and the organisms were demonstrated in the blood and organs.

EXPERIMENT 4.—INULA AND ECHINACEA INJECTED INTRAMUSCULARLY (REMEDIAL)

Three guinea pigs were given daily injections of the "Subculoyd" preparation, the injections being made alternately into the right and left thighs. The dose used was 0.5 mil. After the third injection the animals were all inoculated subcutaneously with 0.5 mil of *Bacillus bovisepticus* culture. On the following day the animals were given a fourth dose of the "Subculoyd." The total dose of remedy was 2 mils. All these cases succumbed to the infection, the first in one day, the second in two days, and the third in three days after the inoculation. The autopsies showed the typical septicemia pictures, and the organisms were demonstrated in the blood.

EXPERIMENT 5.—CONTROLS

Three control animals, each inoculated subcutaneously with 0.5 mil of *Bacillus bovisepticus* culture, all became sick, and two succumbed to the infection. The third survived and after 10 weeks' observation was discharged as recovered. The autopsies on the fatal cases showed septicemia, and the organisms were demonstrated in the blood and organs.

The experiments for septicemia are summarized in Table III.

TABLE III.—*Results of experiments with echinacea in the treatment of septicemia*

Experiment No.	Guinea pig No.	Weight of guinea pig.	Total dose of remedy.	Dose of culture.	Effect.	Termination.	Number of days sick.
		Gm.	*Mils.*	*Mil.*			
1	60	540	4	0.5	Sick	Died	5
	61	500	4	.5	do	do	3
2	65	385	4	.5	do	do	1
	66	335	4	.5	do	do	2
3	62	485	2	.5	do	do	6
	63	505	2	.5	do	Recovered	
	64	385	2	.5	do	Died	12
4	67	355	2	.5	do	do	3
	68	425	2	.5	do	do	1
	69	300	2	.5	do	do	2
5 (controls)	70	380	0	.5	do	Recovered	
	71	365	0	.5	do	Died	3
	72	355	0	.5	do	do	3

SUMMARY OF EXPERIMENTS WITH SEPTICEMIA

"Specific Medicine Echinacea" and "Subculoyd Inula and Echinacea" were used as protective and as remedial measures against septicemia induced by *Bacillus bovisepticus*. The attempt was made to immunize animals against septicemia by administration of the echinacea preparations several days before inoculation. In no case did it appear that echinacea either increased the resistance of the organism to the infection or served to modify it when given as a remedy.

IV.—TESTS OF ECHINACEA AS A REMEDY FOR ANTHRAX

The pathogenic material used to produce anthrax in the experimental animals was a faintly cloudy suspension of *Bacillus anthracis* (48-hour-old agar culture) in sterile normal salt solution. The remedial action of the fluid extract only was investigated, and only five experimental animals were used, the results of the experiment being so definite as not to necessitate the sacrifice of any more animals.

EXPERIMENT 1.—ECHINACEA ADMINISTERED PER OS

Three pigs were given daily doses per os of 2 mils of fluid extract echinacea diluted with 1.5 mils distilled water for 6 days. The total protective dose was 3 mils, equal to 3 gm. of echinacea root. On the eighth day the animals were given per os the same dose of echinacea and immediately afterwards were inoculated with 0.4 mil of anthrax material subcutaneously. On the following day they were given a second remedial dose of echinacea. The total echinacea given was 4 mils of fluid extract. All the animals became sick and all succumbed. No evidence was obtained that echinacea has any influence upon the course of anthrax in experimental animals. The autopsy was typical for anthrax; organisms were demonstrated microscopically in the blood.

EXPERIMENT 2.—CONTROLS

Two controls were chosen at the beginning of experiment 1 of this series and were kept under observation for 8 days, when they were injected subcutaneously with 0.4 mil of the anthrax material at the same time as the experimental animals. Both controls became sick, and one died in 4 and the other in 8 days, having survived the experimental guinea pigs by 1 and 5 days, respectively. The autopsy showed typical anthrax.

Table IV summarizes the results of the experiments for anthrax.

TABLE IV.—*Results of experiments with echinacea in the treatment of anthrax*

Experiment No.	Guinea pig. No.	Weight of guinea pig.	Total dose of remedy.	Dose of culture.	Effect.	Termination.	Number of days sick.
		Gm.	*Mils.*	*Mil.*			
1	73	440	4	0.4	Sick	Died	3
	74	285	4	.4	do	do	3
	75	450	4	.4	do	do	3
2 (controls)	76	355	0	.4	do	do	8
	77	350	0	.4	do	do	4

SUMMARY OF EXPERIMENTS WITH ANTHRAX

Experimental animals were given protective and remedial doses of fluid extract echinacea and were inoculated with *Bacillus anthracis*. All the animals died, those which were treated dying before the control animals. Echinacea does not appear to be of value as a remedy for anthrax.

V.—TESTS OF ECHINACEA AS A REMEDY AGAINST RATTLESNAKE VENIN

Twenty-five guinea pigs were used in the experiments with rattlesnake venin. The venin was furnished by Dr. Park Findley, of Des Moines, Iowa, who had obtained it while with the United States Army on the Mexican border. The venomous secretion of the rattlesnake was collected and dried by inspissation in the sun. This treatment, of course, somewhat attenuated the venin. The product occurred in brittle, clear, yellowish granules, much resembling dried egg albumen. The minimal lethal dose was determined as 2 mgm. for a 400- to 450-gm. guinea pig. The venin was hemolytic in a dilution of 1 to 1,000 against washed sheep corpuscles. For injection, a quantity of the venin was carefully weighed out and dissolved in sufficient sterile normal salt solution to furnish a liquid which would contain 2 mgm. per mil.

EXPERIMENT 1.—ECHINACEA ADMINISTERED PER OS

Each of three guinea pigs received daily 3 mils of a mixture of 1 mil "Specific Medicine Echinacea" and 2 mils water for two doses, a total of 2 mils echinacea, as protective treatment. On the third day the animals were given 2 mgm. of rattlesnake venin in 1 mil of salt solution injected

subcutaneously into the ventral abdominal wall, and immediately afterwards a dose of the echinacea was given per os. No. 50 was found dead on the following morning. The surviving pigs were given a dose of the echinacea mixture. The total amount of echinacea given in the first case was 3 mils; in the second and third cases it was 4 mils. These latter guinea pigs died on the third day after the injection of the venin. All the animals showed the characteristic symptoms and local lesions of this type of poisoning. On autopsy, the characteristic local lesions were found, consisting of a marked inflammatory swelling with necrosis and sloughing of the skin over a considerable area surrounding the point of injection. In cases of early death from rattlesnake poisoning there is usually some oozing of dark, incoagulable blood from the wound at the seat of injection and extensive extravasation of blood into the subcutaneous and muscular tissues. The inflammatory process in most cases extends through the abdominal wall and involves the peritoneum. If the animal survives for several days there may be complete sloughing of the abdominal wall, allowing the viscera to protrude. The internal organs are usually grossly normal in appearance, except in the case of the kidneys, which may be somewhat enlarged and congested with evidence of parenchymatous degeneration.

EXPERIMENT 2.—INULA AND ECHINACEA INJECTED INTRAMUSCULARLY

Each of three guinea pigs received 0.5 mil of the "Subcoloyd Inula and Echinacea" in the right thigh on the first day; on the second day the same dose was injected into the left thigh, both injections being made deeply into the gluteal muscles. The total protective dose was 1 mil. On the third day 1 mil of the venin solution, equal to 2 mgm. of dry venin, was injected subcutaneously into the belly, and immediately afterwards 0.5 mil of "Subculoyd" was injected into the right thigh. On the following day all the animals showed the characteristic symptoms and 0.5 mil of the "Subculoyd" was injected into the left thigh of each animal. The total dose was 2 mils. On the third day No. 51 died; on the fifth day No. 53 died; and six weeks later No. 52 was discharged as recovered. The autopsy was the same as in experiment 1 of this series. The guinea pigs showed the usual local lesions produced by the injection of the inula and echinacea.

EXPERIMENT 3.—CONTROLS

Three guinea pigs were used as controls and were injected subcutaneously into the belly with 1 mil of venin solution, corresponding to 2 mgm. of dry venin. All the controls were sick on the following day. No. 57 and 58 died on the second day and No. 59 on the third day after the injection of the venin. The autopsy showed the same conditions as in experiment 1 of this series. There was no apparent difference between the controls and the treated animals in experiments 1 and 2.

The results are given in Table V.

TABLE V.—*Results of experiments with echinacea as a remedy against rattlesnake venin*

Experiment No.	Guinea pig No.	Weight of guinea pig.	Total dose of remedy.	Dose of venin.	Effect.	Termination.	Number of days sick.
		Gm.	*Mils.*	*Mgm.*			
	31	370	0	0.1	Sick	Recovered	
	32	390	0	.2	do	do	
	33	290	0	.3	do	do	
	34	297	0	.4	do	do	
	35	315	0	.5	do	Died	28
	36	260	0	.6	do	Recovered	
	37	350	0	.6	do	Died	2
A[a]	38	290	0	.7	do	Recovered	
	39	265	0	.7	do	Died	33
	40	440	0	.5	do	do	24
	41	340	0	.5	do	do	28
	42		0	1.0	do	Recovered	
	43		0	1.0	do	do	
	44		0	2.0	do	Died	3
	45		0	2.0	do	do	3
	48	395	4	2.0	do	do	3
1	49	400	4	2.0	do	do	3
	50	370	3	2.0	do	do	1
	51	440	2	2.0	do	do	3
2	52	425	2	2.0	do	Recovered	
	53	360	2	2.0	do	Died	5
	57	385	0	2.0	do	do	2
3 (controls).	58	405	0	2.0	do	do	2
	59	405	0	2.0	do	do	3

[a] To test toxicity of venin.

SUMMARY OF EXPERIMENTS WITH RATTLESNAKE VENIN

"Specific Medicine Echinacea" was administered to guinea pigs per os, and "Subculoyd Inula and Echinacea" was injected as a means of treatment against the venin of the rattlesnake. The venin had been standardized and the minimal lethal dose determined. Neither of the echinacea preparations appeared to influence the course of the poisoning. From these results it does not appear that echinacea is of value in the treatment of rattlesnake poisoning in experimental animals under laboratory conditions.

VI.—TESTS OF ECHINACEA AS A REMEDY FOR TUBERCULOSIS

It has often been asserted that echinacea is a cure for tuberculosis, and for this reason tuberculosis was chosen as one of the chronic diseases upon which to test the remedial value of the plant. The type of organism used to inoculate the experimental animals was strictly human (Igoe strain). The immediate material used for our purpose was one-third of a tuberculous spleen from a guinea pig, third passage of the original material, finely triturated in mortar and suspended in 10 mils of normal salt solution. The dose was 1 mil per guinea pig, injected intraperitoneally.

EXPERIMENT 1.—ECHINACEA ADMINISTERED PER OS

Three guinea pigs were inoculated with tubercle bacilli November 20, 1919, and on the following day treatment was begun. Each animal received a dose of a mixture of 0.25 mil of fluid extract echinacea and 0.75 mil distilled water per os each week day. The animals were weighed three times a week. All the animals showed a progressive loss in weight (see Table VII) and eventually succumbed. Case 85 died December 10, (20 days after inoculation), after having received a total of 3.5 mils of fluid extract echinacea as a remedy. The autopsy in this case was negative. Case 84 died December 22 (32 days after inoculation), having received 6 mils of the echinacea. Case 86 was found dead in the morning of December 26 (36 days after inoculation), having received 6.75 mils of the echinacea. In the last two cases the autopsies revealed the typical picture of generalized tuberculosis.

As these were probably the first experimental animals which had ever received echinacea over an extended period of time, it was interesting to observe the effects of the plant on the animals themselves, and especially upon the gastrointestinal tract. In case 85 there was found a chronic catarrhal gastritis which was absent in cases 83 and 84. Apart from the tubercular lesions there was no abnormality found in the other organs upon macroscopic examination.

EXPERIMENT 2.—INULA AND ECHINACEA INJECTED SUBCUTANEOUSLY

Three guinea pigs were inoculated with the tuberculous material as in experiment 1 on November 20, 1919, and the treatment was begun on the following day. Each animal received subcutaneously 0.2 mil of the "Subculoyd Inula and Echinacea" each week day and was weighed three times a week. All showed progressive loss of weight, as shown in Table VII. Case 87 died December 19, 29 days after inoculation, after having received 4.2 mils of the remedy. Case 88 died December 23, 33 days after inoculation, having received 5 mils of the remedy, and case 86 died on December 28, 38 days after inoculation, having received 5.8 mils of the remedy. The autopsies in these cases showed great emaciation, some necrosis at the points of injection, and generalized tuberculosis. There was no evidence of systemic effects from the remedy.

EXPERIMENT 3.—CONTROLS

Two control guinea pigs were inoculated with the same tuberculous material as the animals in experiments 1 and 2, on November 20, 1919, and were weighed three times a week. They lost weight (see Table VII). Case 89 died December 3, 13 days after inoculation, and case 90 died December 23, 33 days after inoculation. The autopsy showed generalized tuberculosis.

The experiments are summarized in Table VI.

TABLE VI.—*Results of experiments with echinacea in the treatment of tuberculosis*

Experiment No.	Guinea pig No.	Weight of guinea pig.	Total dose of remedy.	Effect.	Termination.	Number of days sick.
		Gm.	*Mils.*			
1	83	430	6.75	Sick	Died	36
	84	425	6.00	do	do	32
	85	495	3.50	do	do	20
2	86	470	5.80	do	do	38
	87	450	4.20	do	do	29
	88	405	5.00	do	do	33
3 (controls)	89	425	0	do	do	13
	90	550	0	do	do	33

TABLE VII.—*Progressive loss of weight of guinea pigs in experiments with echinacea in the treatment of tuberculosis*

Date.	Weight of guinea pigs treated with fluid extract echinacea.			Weight of guinea pigs treated with "Subculoyd Inula and Echinacea."			Weight of control guinea pigs.	
	No. 83.	No. 84.	No. 85.	No. 86.	No. 87.	No. 88.	No. 89.	No. 90.
1919.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Nov. 18	430	425	495	475	450	405	425	550
20	410	430	480	455	450	405	415	535
22	395	410	470	440	420	380	405	520
24	405	410	490	460	425	360	395	530
26	400	415	475	460	415	370	370	525
28	390	415	480	470	415	370	325	525
Dec. 1	385	390	475	470	395	355	285	510
3	380	395	460	470	405	360	265	510
5	365	385	440	460	375	340	.....	510
8	355	365	405	465	360	335	.....	485
10	350	355	.....	460	355	325	.....	485
12	345	345	.....	445	325	330	.....	480
15	310	325	.....	405	285	300	.....	400
17	295	290	.....	405	260	280	.....	380
19	290	270	.....	400	.....	280	.....	355
22	270	.....	.....	355	.....	245	.....	305
24	240	.....	.....	340	.....	.....	.....	.....
26	.....	.....	.....	320	.....	.....	.....	.....

SUMMARY OF EXPERIMENTS WITH TUBERCULOSIS

Fluid extract echinacea was administered per os, and "Subculoyd Inula and Echinacea" was injected subcutaneously into experimental guinea pigs daily for extended periods as remedies for tuberculosis produced by a human type organism. Neither of the preparations appeared to influence the course of the disease. From these results it does not seem probable that either fluid extract echinacea or the "Subculoyd Inula and Echinacea" is of value in the treatment of tuberculosis.

The experimental animals did not show organic effects from echinacea ingested in large doses for a long time.

VII.—TESTS OF ECHINACEA AS A REMEDY FOR TRYPANOSOMIASIS (DOURINE)

In connection with the experimentation with tuberculosis it was considered of interest to study the remedial action of the echinacea preparations upon another chronic condition. Trypanosomiasis induced by *Trypanosoma equiperdum* and commonly called dourine was chosen. This disease as produced under laboratory conditions in guinea pigs runs a course of from 7 to 11 weeks, allowing ample time for the exhibition of quantities of remedial agents and consequently favoring the remedy more than a speedy, acute infection would.

The material used to produce the disease was kindly furnished by Dr. H. W. Schoening, of this laboratory. It consisted of a normal salt suspension of a sample of blood freshly drawn from rats which had been inoculated three days previously with *Trypanosoma equiperdum*. Upon microscopic examination this showed numbers of trypanosomes. The dose given was 0.5 mil, injected subcutaneously.

EXPERIMENT 1.—ECHINACEA ADMINISTERED PER OS

Three guinea pigs were inoculated with the dourine material on December 1, 1919. On the following day treatment was begun, each animal receiving 1 mil of a mixture of 0.25 mil fluid extract echinacea and 0.75 mil of distilled water. This dose was given each week day thereafter as long as the animal survived. All the animals were weighed three times a week. The weights are reported in Table IX. At intervals the blood of some of the animals was examined microscopically for the presence of trypanosomes; on December 17 these were demonstrated in the peripheral circulation of case 93, on January 6 in that of case 92, on January 16 in cases 91 and 92, and on March 3 in case 93. All the animals showed the typical symptoms of trypanosomiasis. Case 91 died on the sixty-first day, after having received 12.5 mils of the fluid extract; case 92 died on the sixty-fourth day after having receiving 13 mils of fluid extract; case 93 died on the ninety-third day, after having received 15.75 mils of fluid extract. Treatment of case 93 was suspended February 14. The autopsies showed the usual picture of this type of infection. In case 91 there was a chronic catarrhal gastritis; otherwise no organic effects from the extended ingestion of the echinacea were discovered.

EXPERIMENT 2.—INULA AND ECHINACEA INJECTED SUBCUTANEOUSLY

Three guinea pigs were inoculated and treated exactly as in experiment 1, except that the remedy given was 0.2 mil of the "Subculoyd Inula and Echinacea" each week day. The weights of the animals are given in Table IX. On December 17 trypanosomes were demonstrated in the peripheral circulation of case 94, on January 6 in that of case 96, and on

January 16 case 96 was positive, while cases 94 and 95 did not show trypanosomes. Case 96 died on the forty-eighth day after inoculation, having received 7.8 mils of the remedy; case 95 succumbed on the sixty-sixth day, after receiving a total of 10.8 mils of the remedy, and case 94 died on the seventy-first day, having received 11.4 mils of the remedy. The autopsies in these cases showed a dirty, dark discoloration of the subcutaneous and superficial abdominal muscular tissues over the area where injections were made. Extreme emaciation was evident, the spleen was greatly enlarged, and in general the typical dourine picture was present.

EXPERIMENT 3.—CONTROLS

Four guinea pigs were used as controls. These were inoculated on the same date as those in experiments 1 and 2 and were kept in separate cages. One animal died in 17 days, another died in 30 days, and the remaining 2 died in 78 and 79 days, respectively, all with typical dourine symptoms.

These experiments are reported in Table VIII.

TABLE VIII.—*Results of experiments with echinacea in the treatment of dourine*

Experiment No.	Guinea pig No.	Weight of guinea pig.	Total dose of remedy.	Effect.	Termination.	Number of days sick.
		Gm.	*Mils.*			
1	91	475	12.5	Sick	Died	61
	92	505	13.0	do	do	64
	93	445	15.75	do	do	93
2	94	435	11.4	do	do	71
	95	400	10.8	do	do	66
	96	560	7.8	do	do	48
3 (controls).	97	535	0	do	do	17
	98	460	0	do	do	30
	99	535	0	do	do	78
	100	500	0	do	do	79

TABLE IX.—*Progressive loss of weight of guinea pigs in experiments with echinacea in the treatment of dourine*

Date.	Weights of guinea pigs treated with fluid extract echinacea.			Weights of guinea pigs treated with "Subculoyd Inula and Echinacea."		
	No. 91.	No. 92.	No. 93.	No. 94.	No. 95.	No. 96.
1919.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Nov. 29	475	505	445	435	400	560
Dec. 1	475	505	450	445	410	570
3	485	510	465	455	415	585
5	475	500	465	440	425	560
8	480	505	470	445	420	555
10	480	510	475	450	415	570
12	500	520	505	445	440	565
15	495	515	500	450	425	565
17	485	510	500	445	430	565
19	490	520	525	455	445	550
22	485	510	490	410	460	560
24	465	505	475	410	435	535
26	450	495	470	430	440	495
29	460	480	465	420	435	500
31	460	485	465	405	430	485
1920.						
Jan. 2	450	470	470	410	445	480
3	455	475	470	420	455	480
5	465	485	500	435	465	480
7	450	470	485	415	450	470
9	455	465	500	430	460	460
12	435	455	510	445	465	435
14	415	435	495	420	440	395
16	410	430	500	410	450	365
19	395	435	520	365	465	.....
21	385	415	505	340	445	.....
23	400	415	515	325	440	.....
26	390	385	510	305	440	.....
28	385	355	515	315	440	.....
30	.....	320	500	315	450	.....
Feb. 2	.....	.....	495	295	405	.....
4	.....	.....	480	295	.....	.....
6	.....	.....	490	305	.....	.....
9	.....	.....	455	.....	.....	.....
11	.....	.....	440	.....	.....	.....
13	.....	.....	450	.....	.....	.....
16	.....	.....	455	.....	.....	.....
19	.....	.....	455	.....	.....	.....
21	.....	.....	415	.....	.....	.....
24	.....	.....	390	.....	.....	.....
27	.....	.....	370	.....	.....	.....
Mar. 1	.....	.....	345	.....	.....	.....

SUMMARY OF EXPERIMENTS WITH DOURINE

Fluid extract echinacea and "Subculoyd Inula and Echinacea" were tested as remedies in trypanosomiasis (dourine). Neither of these preparations appeared to influence the course of the disease. They certainly have no curative value.

GENERAL SUMMARY

Various preparations of echinacea—namely, the "Specific Medicine Echinacea," the fluid extract echinacea, and the "Subculoyd Inula and Echinacea"—were studied as remedies in several types of infectious and allied diseases, both acute and chronic, in guinea pigs.

In both tetanus and botulism produced by the administration of bacterial toxin the course of the disease was not modified by the echinacea.

In septicemia produced by injection of a culture of *Bacillus bovisepticus*, and in anthrax produced by injection of *B. anthracis* the results indicated that echinacea had no influence.

In poisoning by the venin of the rattlesnake produced by injection of a solution of the dry venom the echinacea preparations were without curative effect.

In the chronic diseases, tuberculosis produced by injection of a human strain of the bacillus and trypanosomiasis produced by injection of *Tryponosoma equiperdum* the remedy was exhibited over an extended period of time without apparently influencing the course of these diseases.

Definite evidence of organic effects from the echinacea itself was not obtained.

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